ANNALES MEDICINAE EXPERIMENTALIS ET BIOLOGIAE FENNIAE

STUDIES OF THE VITAMIN A AND C STATUS AMONG PRISONERS, SOLDIERS AND CIVILIAN POPULATION IN FINLAND

BY

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VOL. 27 1949 SUPPLEMENTUM 3

MERCATORIN KIRJAPAINO, HELSINKI 1949

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ACADEMIC DISSERTATION TO BE PRESENTED WITH THE ASSENT OF THE MEDICAL FACULTY OF THE UNIVERSITY OF HELSINKI FOR PUBLIC EXAMINATION IN AUDITORIUM XII ON SEPTEMBER 14th, 1949, AT 12 O'CLOCK

HELSINKI 1949

Also published as a Supplement 3 to Vol. 27 of
Annales Medicinae Experimentalis et Biologiae Fenniae
1949

PRINTED IN FINLAND BY PAINOTEOLLISUUS OY
Helsinki 1949

PREFACE

This research was launched in 1939 at the suggestion of Professor P E. S i m o l a, M. D., the Chief of the Department of Medical Chemistry of the University of Helsinki. It was protracted, because owing to the wars, I was compelled to suspend experimental work several times and partly change my plan of work.

I am greatly indebted to Professor P. E. S i m o l a for his unflagging interest in my work and the valuable advice he gave me when I was studying the methods for determining vitamins and throughout the course of this research.

Further, I wish to express my gratitude to Lieutenant Colonel V. N o r d l u n d and Surgeon Lieutenant Colonel J. T i i t t a n e n for the interest they took in the investigations I made on the soldiers under their command.

I also beg to thank Mrs. A. L. R e p o, M. A., and Mrs. H. P a l a s v i r t a, M. A., for their English translation of my study, as well as all the persons who in one way or another have aided me in this research.

VEIKKO J. UUSPÄÄ.

January, 1949. Helsinki, Laivastokatu 14.

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INTRODUCTION

In Finland and in general in all the countries of the northern latitudes the one annual crop is collected, owing to the short summer season, within a relatively short period, two or three months. Thus obtaining the most varying and quite fresh nourishment is limited to those early autumn months. The people of the northern countries are compelled to use nutriment preserved in one form or another over a shorter or longer period.

Many products of the vegetable kingdom suitable for human nourishment are difficult and expensive to preserve. Consequently their production is limited. The number of the products which are intended to satisfy the demand until the next crops is very small, primarily comprising grain and the potato. Therefore, in the cold countries the nourishment provided by the vegetable kingdom is for a great part of the year, in addition to being in preserved form, also rather unvaried.

Recent nutritional research has demonstrated that many products of the vegetable kingdom lose a considerable proportion of their nutritional value when preserved although they appear to keep well. It has been observed that this loss pertains particularly to the vitamins. Vitamin C is especially sensitive to preserving. For example, the potato in storage from autumn to spring loses 50—75 % of its vitamin C content which may be as high as 30 mg %, and the pea has lost its whole relatively high, 40 mg %, vitamin C content by the time it is completely dried.

As the vitamins are substances which the organism is unable to produce but which must be obtained from outside ready in nourishment, it is easily comprehensible that the vitamin reserves of the organisms of the northern peoples are subject to fluctuations in different seasons.

Since, as is known, the human organism is incapable of storing any great quantity of vitamin C or of synthesizing it, it can be expected that the decrease of vitamin C intake during the winter may result in a considerable decrease of the reserves of the said vitamin in body. In fact, in Finland v. B a g h ('38) with respect to the spinal fluid, R a u r a m o ('41) and K i n n u n e n and R a u r a m o ('45) with respect to the placenta, and R a u n i o ('41), P i t k ä n e n ('44), W i n g r e n ('45, '46) and T ö t t e r m a n ('49) with respect to serum have found that the vitamin C content is lower in the spring and early summer than in the autumn.

The animal organism has a considerably greater capacity for storing vitamin A, primarily in the liver, which contains ca. 95 % of the body's total vitamin A reserves (M o o r e '31, D a v i e s and M o o r e '34, W i t h '41, etc.). These vitamin reserves, if normal in quantity are sufficient to satisfy the requirements, although obtaining replenishment should for some time become difficult or stop completely. The liver's capacity of storing vitamin A evidently depends also upon other factors than the vitamin A content of the nourishment. The matter, however, requires further research. S u o m a l a i n e n ('40) discovered that intense storing of vitamin A takes place in the liver of the hedgehog immediately before hibernation. I k e g a k i ('39) made the observation that the several organs of an animal have a fairly limited capacity of storing vitamin A.

Food-stuffs when preserved do not lose as great a proportion of vitamin A as of vitamin C. Besides, in Finland milk products are the chief source of vitamin A and constitute the fresh part of the diet also in the winter. Yet, the vitamin A content of the nourishment of the Finnish people is not constantly the same and sufficient in every season of the year. The vitamin A content of milk and milk products is reduced, by spring, to nearly one-third of what it is during the cattle's grazing time, because the feed for the cattle's indoor period is still mainly, despite the increasing production of AIV-feed, preserved by drying, which reduces its carotene content as the winter progresses (V i r t a n en '38, V i r t a n e n and T u r p e i n e n '40, W i l l s t a e d t '41, S k u r n i k and H e l l é n '44.) Since the production of edible plants containing carotene is insufficient, and the preserving methods as yet unsatisfactory, the milk products are

even to a greater extent in the spring the main source of vitamin A. Consequently the Finnish people's nourishment, even in normal times, weakens as to vitamin A content towards the spring. According to investigations of the Kansanravitsemuskomitea [People's Nutrition Committee (PNC)] ('40) 94 % of the poor families received less vitamin A than the requirement value implies.[1]) But particularly during the war and also post war because of general food rationing and because emergency conditions to the greatest extent limit the supply of the food-stuffs which are the chief source of vitamin A, the decrease of the vitamin A content of the nourishment appears to be so considerable that one must take into consideration the exhaustion of the vitamin A reserves with the attendant symptoms characteristic of vitamin A deficiency. In his investigations S i m o l a ('41) observed that 25—35 % of the children of poor families had decreased dark adaptation in the spring.

As to the other vitamins, it appears that under normal conditions the Finnish people's nourishment contains sufficient quantities of the most known components of the vitamin B complex [K a n s a n r a v i t s e m u s k o m i t e a (P e o p l e's N u t r i t i o n C o m m i t t e e) '40, S i m o l a '41, P i t k ä n e n '44]. S i m o l a has particularly emphasized the fact that vitamin D deficiency conditions are at the forefront in Finland. As a matter of fact, even today the rickets is a regrettably common disease among the Finnish children (R u o t s a l a i n e n '22, Y l p p ö '25, L y y t i n e n '35, L e p p o '40).

Under exceptional conditions, when there is a scarcity of fats and meat in particular, a great variety of deficiency conditions are apt to develop. It is a well-known fact that the consumption of vitamin B_1 increases considerably when the increased requirement for energy nourishment must be satisfied exclusively by carbohydrates, which may lead to hypovitaminosis-B_1). Moreover, as is also known, conditions similar to pellagra easily develop under an exclusive diet of grain and peas (S t a n n u s '11).[2])

[1]) The PNC considered that an adult requires 4,000 and a child (under 12 years) 2,000 international units of vitamin A daily.

[2]) The author verified during the last war several cases of pellagrous dermatitis, which cured quickly with injections of nicotinamide.

Furthermore, it has been observed in tests on animals that in vitamin A deficiency the weakening of the capacity for vitamin C synthesis and of the effect of vitamin C, as well as decrease in the vitamin C content in the organs and even changes resembling those in scurvy have developed as secondary phenomena (Sure, Theis and Harrelson '39, Jonsson, Obel and Sjöberg '42, Moore '46).

Numerous questions relating to vitamin metabolism are still subject to dispute. The daily human requirements of the several vitamins are of great importance among them. The investigators are of divergent opinions particularly with respect to the optimal requirement values of the vitamins. Further, the questions of what is the normal vitamin C content of the blood and where the limit between normal and pathological shall be drawn are open to dispute. Since in the human being the blood is primarily the tissue from which it is easy to take a specimen for examination, it is important that a final and indisputable answer to these questions be obtained. Not until then can the optimal requirement values — i.e. the quantity of vitamin C per day which is required to maintain the optimal vitamin C concentration of the blood for individuals of different ages in different circumstances be determined. And by means of test series it could be ascertained to what extent during the different seasons of the year and for how long a time within a year the population of a given country lives with respect to vitamin C under such circumstances which cause diseases, and then the proper measure could be taken for improving the unfavourable conditions.

Moreover, it is of importance to discover to what extent the requirement values determined by research among the population of another country could be utilized in judging nutritional conditions in Finland. The geographical and climatic conditions under which the Finnish people live and the in many respects unique habits which they have adopted doubtless have a deep effect on metabolism.

It is noteworthy that qualitatively too the appetite varies with the seasons: in the winter we require butter and pork, and in the summer we crave for fatless foods, such as buttermilk and berries, which taste good during that season and seem to satisfy our appetites. The effects of variations in temperature seem to penetrate deep into cell-life. Besides the variations in temperature, also other

climatic factors, such as before all sunlight, have their effects on vitamin metabolism. We know that sunlight has actually the same effect as vitamin D.

Consequently it may be concluded that in Finland, owing to the effect of the long, cold and dark winter, the people's vitamin requirements are subject to fluctuations with the seasons and that they thus at certain periods may differ from that of the people living in southern countries. In this connection particular attention must be paid to the considerable length of the cold season, which involves reduced vitamin supply but simultaneously most likely also increased consumption of vitamins[1]), in contrast to the brevity of the crop season, which means plentiful vitamin supply.

What has been presented above with respect to the variations in the vitamin content of the Finnish people's nourishment during the different seasons of the year naturally pertains, and to a particularly great extent, to the nourishment of prisoners in Finland. Although in planning the dietary for prisons health aspects, alongside costs, have been given special consideration, it is nevertheless evident that it is difficult, even in normal times, to make the prisoners' diet as varying and satisfactory to individual tastes and requirements as that of the free population. It is particularly difficult to include as great quantities of the food-stuffs containing vitamin A, such as butter and milk, in the prison dietary as many prisoners have been accustomed to consume when free. The food rationing of emergency conditions falls more heavily on the prisoners than on the free population, who have greater possibilities of obtaining variation in and additions to their rationed diet. Therefore it must be taken into consideration that deficiency diseases may occur in the prisons even in normal times and especially in times of shortage of supplies.

As the number of prisoners in Finland amounts to the population of a parish of average size and the yearly exchange of prisoners is considerable, the hygienic conditions of the prisons and the general condition of the prisoners possess significance from the point of view of public health. Since, to my knowledge, in Finland the

[1]) Kyhos *et al.* ('44) have ascertained that the quantity of ascorbic acid (50 mg. daily) which in the summer was sufficient to maintain the optimal ascorbic acid concentration of the blood plasma was insufficient for maintaining it in the winter and spring. Cf. also Sheahan '47.

vitamin condition of the prisoners has not been scientifically studied, I in my capacity of prison physician have considered it motivated to make such a study with respect to vitamin A and C status among them.

The history of wars and wartime medical literature reveal that armies have invariably been especially disposed to deficiency diseases, whose occurrence has at times been similar to an epidemic, despite the fact that nations have put forth all their efforts to provide them with food supplies and other commodities. In view of the special significance during wartime of the general health and physical condition of the soldiers and of the special demands war makes on medical service, it is evident that one of the functions of a military surgeon in wartime is to keep an alert eye on the soldier's general condition. With this in mind during the last war I studied the vitamin status of soldiers in my unit.

PART I

STUDIES ON VITAMIN A

CHAPTER I

ON THE SYMPTOMATOLOGY OF VITAMIN A DEFICIENCY AND PARTICULARLY HEMERALOPIA AS AN EARLY SYMPTOM

The lack of vitamin A in nourishment causes a systemic disease of the epithelial tissue (Wolbach and Howe '25, '33, Pillat '35). Foremost in the complex of symptoms is a specific eye syndrome, whose early symptom is hemeralopia. As the deficiency state advances xerophthalmia and keratomalacia occur.

The relation of vitamin A to hemeralopia has been the subject of numerous studies, only a part of which can be referred to here. Holm ('25) demonstrated that in rats a diet lacking vitamin A caused night blindness, which was cured by vitamin A. In rats kept without vitamin A visual purple regeneration took place more slowly (Friedericia and Holm '25) and less of it was formed than under normal conditions (Tansley '31). Granit ('38, '39) and Charpentier ('36) have confirmed these results.

The importance of vitamin A in night vision became increasingly evident when Wald ('35, '38) succeeded in demonstrating that visual purple is a conjugated protein whose prosthetic group is a carotene-like substance retinine, and that this substance in the isolated, light-bleached retina of a frog was destroyed into vitamin A. "The light sensitivity of the visual cells is undoubtedly related to the particular prosthetic or chromophore group in the rhodopsin (visual purple) molecule" (Hecht '42).

Morton and his collaborators ('44, '48) have shown that the chromophore group in the rhodopsin molecule is a vitamin A aldehyde, which can be obtained by oxidizing from vitamin A alcohol or β-carotene (Hunter and Hawkins '44, Hunter and Williams '45) or by synthesizing from β-ionone (Arens and van Dorp '46, '47, Milas '46, Karrer *et al.* '46, etc.). The vitamin A alcohol itself does not form a part of the rhodopsin molecule, although it is necessary for the formation of rhodopsin.

According to Granit ('43, '47) "the A vitamins are, no doubt, of importance for the formation of the chromophoric group of the visual purple molecule, even though it may not be possible at present to describe the nature of this relationship".

It has been demonstrated experimentally that vitamin A deficiency caused in a human being night blindness, which was cured by vitamin A (Jeghers '37, Wald, Jeghers and Arminio '38, Wald and Steven '39, Booher, Callison and Hewston '39, Steffens, Bair and Sheard '39, v. Drigalski '39, Wagner '40, Blanchard and Harper '40, Batchelder and Ebbs '44, etc.). The period of latency during which the hemeralopia developed varied from 1 to 124 days and no doubt was at least partly dependent upon the subject's vitamin A status at the start of the experiment. Also the time in which administration of vitamin A restored dark adaptation to normal varied, according to the different investigators, from a few minutes to months (Edmund and Clemmesen '36, etc.).

Hemeralopia, however, is not specific to vitamin A deficiency. Congenital hemeralopia is a very rare hereditary adaptation anomaly without local eye changes. Dieter ('29) discovered 8 cases thereof among 200,000 eye patients. Photopic and colour vision were normal, but night vision was completely absent. This type of hemeralopia is total and stationary. In Dieter's cases vision in the maximal adaptation state varied 3,200—6,600 microlux. Also certain eye diseases, such as opacities of the cornea, the vitreous humor or the lens, refraction anomalies of high degree, anomalies of the pupil, retinitis pigmentosa, ablatio retinae, glaucoma, retinitis albuminurica, chorioiditis disseminata, chorioiditis syphilitica, atrophia nervi optici and some other even rarer eye diseases may result in the weakening of the night vision. Moreover in some kinds of intoxications, such as those caused by alcohol, quinine, carbon disulphide, nicotine, adrenaline and several war gases, as well as often in connection with liver diseases, hemeralopia may occur, but the cause of same even in these cases may be disturbances in the vitamin A metabolism (according to Lindqvist '28). However, those cases must be excluded before hemeralopia may be diagnosed as caused by vitamin A deficiency.

Besides the visual action of the retinal rods, also that of the cones appear to be to some extent dependent upon vitamin A. In vitamin A deficiency the threshold values also in cone vision increase (Hecht and Mandelbaum '40). Morton ('44) stated that "vitamin A deficiency not only delays dark adaptation in scotopic (rod) vision but also very definitely affects photopic (cone) vision, and that vitamin A is concerned in both mechanisms".

In the later stages of vitamin A deficiency there occur, particularly in a growing organism, besides changes in the visual cells of the retina, also drying, keratinization, disappearance of nuclei and pigmentation in the scleral conjunctiva (xerosis conjunctivae bulbi, Bitot's spots), softening of the cornea and even perforation thereof (keratomalacia) as well as keratinization of the lachrymal and Meibom's glands (Pillat '35, Sweet and K'Ang '35, Nicholls and Nimalasuriya '39). Through meta-

plasia of the epithelium the conjunctiva becomes thick and wrinkled with the result that the patient may have the sensation of having sand or some other foreign body in the eyes. Irritation causes photophobia, lachrymation and conjunctival injection and thus the disease may resemble inflammatory conjunctivitis. A metaplastic epithelium in fact, is disposed to secondary infection, which causes softening, ulceration, often even perforation of the cornea and thus complete loss of vision (Blegvad '23). But a considerably opaque cornea can be clarified by administration of vitamin A orally or parenterally (Blegvad '23, Bloch '25, personal observation). Also local application of vitamin A appears to increase the vitality of the corneal cells. The deficiency symptoms usually occur simultaneously in both eyes, but frequently in different degrees of intensity.

The epithelial changes in vitamin A deficiency are not limited exclusively to the eyes and the lachrymal glands but extend to all the organs having epithelium. Already at an early stage the skin becomes dry, rough and desquamated, which is soon followed by an at first localized, but most frequently quickly spreading, dry, keratinized papulous dermatitis, follicular hyperkeratosis (Pillat '29, '35, Frazier and Hu '31, Nicholls '33, Loewenthal '33, Goodwin '34, Steffens *et al.* '39, Abbott *et al.* '39, etc.). Hyperpigmentation of the skin is also observed. The hair frequently becomes dry, lustreless and brittle. The nails likewise become brittle. Also the mucous membranes become dry as their glandular cells become atrophied. Clinical symptoms of dry rhinopharyngitis and laryngotracheobronchitis and xerostomia, hypacid or anacid gastritis and enterocolitis follow. Keratinization phenomena occur also in the epithelium of the urogenital apparatus, the renal pelvis, the bladder and the urethra, the uterus and the vagina (Pillat '35). The change in the epithelium of the vaginal mucosa is such a typical and constant symptom that it is used for ascertaining avitaminosis-A in animal tests.

As regards the histology of these epithelial changes, the first changes are observable in the basal cells, whose division and reproduction accelerates and which begin to form keratinized pavement-epithelium, independently of the structure of the original epithelium (Wolbach and Howe '33). In Finland Järvi ('38) has studied changes produced in the epithelium of the salivary glands in lack of vitamin A and he has observed in the larger gland canals typical pavement-epithelium metaplasiae and keratinization of the epithelium, as well as atrophy of the glandular cells. The cell components, as the nucleus, mitochondria, Golgi's apparatus, retained their normal structure.

Kalaja ('39) has observed bone changes in rats kept without vitamin A. The changes appeared already on the twentieth day of vitamin A deficient diet, before the rats stopped growing. Kalaja observed qualitatively analogous phenomena also when the rats were kept without the vitamin B complex and in hunger. Mellanby ('41, '43, '47) has observed in growing animals disturbances in the function of the osteoclasts and osteoblasts, thickening and dysplasia of the bones, which may cause degenerative pressure injuries in the brain and cranial and peripheral nerves.

Moreover, changes have been observed in the teeth as a result of vitamin A deficiency. In a far-advanced state of deficiency and particularly in growing teeth the ameloblasts diminish in size and finally disappear entirely, the result of which is atrophy of the enamel and its complete destruction in some places (Simola '32, Pohto '38, Wolbach and Howe '33, '38). But prior to the enamel changes, from the fourth week on atrophy of the odontoblast layer, disturbances in the formation of dentine and calcification are observed (Pohto). Jonsson *et al.* (l.c.) assert that these mesenchymal changes are due to secondary scurvy caused by vitamin A deficiency.

In experimental vitamin A deficiency in human beings Wagner (l.c.) ascertained from the fifth month on changes also in the blood: slight hyperchromic anemia, anisocytosis and poikilocytosis, leucopenia and thrombopenia and prolongation of the coagulation time. The Rumpel-Leede test was positive. The vitamin A gradually disappeared entirely from the blood. All of these symptoms as well as hemeralopia cured entirely when the subjects were given 2,500 I.U. of vitamin A or 5,000 I.U. β-carotene daily.

Abbott *et al.* ('39) have observed both in animals and human beings with vitamin A deficiency changes in the white blood picture: slight leucopenia with a decrease in polymorphs, a relative increase in large lymphocytes, occurrence of degenerate cells, particularly granulocytes and immature cell formations. These changes disappeared in some weeks after large doses of vitamin A were administered.

Several investigators state that there is a correlation between dark adaptation and the vitamin A concentration of the blood plasma (Lindqvist '38, Saksela '40, Pett and Le Page '40, etc.), whereas Yudkin ('41) regards the vitamin A determinations in the plasma as insufficient for the diagnosing of mild vitamin A deficiencies. Young and Wald ('40) claim that the vitamin A concentration of the plasma varies also because of other factors than in relation to the vitamin A reserves of the body. The body seems to maintain a certain constant vitamin A concentration of the blood until its own reserves are exhausted. Storing of vitamin A is also dependent on the vitamin E status (Moore '40, Davies and Moore '41).

Lindqvist's, Saksela's, Wagner's and others' studies reveal that when the vitamin A concentration of the blood plasma is reduced below a certain limit which, however, varies considerably according to the individual, hypovitaminosis-A is concerned, the first observable clinical symptom of which is night blindness or hemeralopia. And it is hemeralopia which has been used as a criterion in judging whether the vitamin A concentration of the blood plasma is normal or pathological. "Da die Hemeralopie das frühest nachweisbare Symptom der Hypovitaminose A darstellt, muss ein für den normalen Verlauf der Dunkeladaptation genügenger Vitamin A-Gehalt in Serum als Normalwert in dem Sinne betrachtet werden, dass er keinen sicher pathologischen Wert darstellt. Ein Vitamin A-Gehalt im Serum, der eine Hemeralopie mit sich führt, muss dagegen als pathologisch bezeichnet werden" (Lindqvist, l.c., p. 123). Lindqvist deems adaptometry an especially appropriate method for ascertaining hypovitaminosis-A in mass-examinations.

CHAPTER II

AUTHOR'S INVESTIGATIONS

1. *Material*

The first group of the material comprises the results of the examinations which I performed at the initial stage of the research in the summer 1939 among the prisoners of the Reserve Prison at Karvia. After the interruption caused by the war between the Soviet Union and Finland 1939—40 I continued collecting material at the said prison. For comparison material I performed in 1941 dark adaptation tests on primary school children in the rural community of Karvia, and I also performed dark adaptation and vitamin A and C estimations on healthy and sick subjects.

After my having been summoned again in June 1941 to serve in the Defence Forces my attention was drawn to the vitamin status of the soldiers. But not until the end of the first stage of the Fenno-Russian war, in September 1941, did I get an opportunity as the surgeon of the Coastal Artillery Regiment in the town of Käkisalmi to undertake a systematic study of the vitamin status of soldiers. I performed my examinations at the hospital of the said unit. The material consists of tests on healthy soldiers and soldiers being treated at the said hospital for various diseases. To obtain material for comparison I made corresponding tests on the civilian population of the town of Käkisalmi and its environs.

My material comprises the following groups of subjects:

Prisoners:	male,	examined	in the year	1939	129	
	„	„	„ „ „	1941	134	
	female	„	„ „ „	1945	62	325
Soldiers		„	„ „ years	1940—43		245
Primary School Children		„	„ „ year	1941		100
Other Subjects		„	„ „ years	1040—43		80
					Total	750

2. *Methods*

In the present work I have used for measurement of dark adaptation the biophotometer[1]) presented by Jeans, Blanchard and Zentmire ('37), which is the apparatus that has most commonly been used in Finland in studies of the vitamin A status (Leppo '39, Saksela '39, '40, Simola and Saksela '40, '41, Pitkänen '44). The biophotometer has been described in details by Saksela ('40) in his dissertation.

In its original form the method of Jeans *et al.* has been criticized considerably (Palmer and Blumberg '37, Nylund '44, etc.). It appears, however, that it is rather the interpretation of the results than the instrument that has been criticized. Lindqvist ('38) remarks that obviously the scope of the normal has hitherto been estimated as too small, because all the subjects do not, even after the use of vitamin A over a long period, attain normal values. He also considers it doubtfull, whether so much weight should be given, as many investigators have done, to the perception value obtained directly after exposure to bright light, because according to the prevailing opinion perception of light during the first minutes takes place by means of the cones, which do not contain visual purple and whose function cannot therefore be so directly related to vitamin A as that of the rods.

Nylund ('44) ascertained that 85 % of the persons suffering from night blindness whom he examined had a normal threshold value subsequent to dark adaptation lasting 30 seconds, and that also normal individuals, although in rare cases, had increased threshold values after 30 seconds had elapsed. Nylund drew the conclusion that the threshold value obtained after 30 seconds dark adaptation could not be used as a criterion for ascertaining night blindness. Nylund in an extensive paper presents the view that only the threshold values obtained after 16—20 minute periods of dark adaptation can indicate with certainty whether night blindness is present or not. But he deems that prolonging the adaptation period over 20 minutes is not to any further advantage, although dark adaptation has not yet come to a close within that period.

Nylund presents the following criticism in relation to the biophotometer: "Die Anordnung für Helladaptation des Biophotometers gestattet aber nur Beleuchtung eines kleinen Teiles der Netzhaut, was wahrscheinlich als ein Nachteil anzusehen ist." This fact may possibly have an effect on the threshold values of the initial part of the dark adaptation curve but not on the reliability of the final results if for a criterion the threshold values of the terminal part of the dark adaptation curve which are obtained in maximal or almost maximal dark adaptation are used.

Lindqvist ('38) states with respect to the biophotometer that it is evidently partly used for examining the adaptation of cones, about whose relation to vitamin A nothing is known. This remark, doubtless, does not pertain to the instrument itself.

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[1]) Manufactures Frober — Faybor Company, Cleveland, Ohio.

As 10 minutes is obviously too short a period for measuring the dark adaptation capacity, I have in this research[1]) continued testing the dark adaptation at 2 $^1/_2$ minute intervals until the threshold values have, practically viewed, remained the same in 2—3 readings successively. I have determined, in addition to the threshold values for the central dot of light, also the threshold values for the left and right pairs of dots of the total of five dots, that is always for the dot appearing last. In that way I have been able to eliminate the subject's possible mistakes with respect to the dots. Otherwise too the method has added to the reliability of the results. A reading was made for the threshold value of the second dot about 15 seconds prior to the reading of the central dot and for the threshold value of the fifth dot about 15 seconds after the reading of the central dot. The whole test, which otherwise was made in the way described by Saksela (l.c.), lasted about 35—45 minutes including the beginning (10 min.) and illumination period (3 min.).

The threshold values have been given in 1/1000 millifoot candles i.e. microfoot candles (μfc.). 1 μfc. = 1.076 micromillilambert = about 1/100,000 lux.

In order to be able to judge on the basis of the values obtained by the biophotometer whether dark adaptation is normal or whether night blindness is involved, one must know what threshold values in general are to be considered normal and what threshold value indicates the limit between normal and pathological night vision when this method is used. To discover this I made the test on a number of persons of different ages and subjectively healthy, both children and adults.

Group I comprises 100 pupils of the primary school of Karvia church village, 53 of them boys and 47 grils. This group of pupils was not chosen either on the basis of the condition of health or a dietary history, but every pupil who was present at school was examined. They were all subjectively healthy. Under the conditions prevailing then the natural vitamin A status of the pupils could not be improved by administering vitamin A to them despite the fact that the time, the spring of 1941, when the tests were made was not by

[1]) In 1939 I made the dark adaptation measurements of 129 prisoners according to the original Jeans, Blanchard and Zentmire method (See p. 30).

TABLE 1

Averages of Threshold Values Obtained after Periods of Dark Adaptation of 30 Seconds to 30 Minutes for Pupils of the Primary School of Karvia Church Village in 1941.

Dark Adaptation Period in Min.	M_{100}	$M_{100}+3\sigma$	M	$M+3\sigma$	M_7	v
½	519 ± 28.6	1377	502.7 ± 29.8	1364.6	734.6	57.2
2½	108 ± 7.6	346	102.1 ± 7.7	324.1	181.0	72.5
5	36 ± 3.6	143	32.3 ± 3.2	125.1	85.9	95.7
7½	16.7 ± 2.0	75	14.3 ± 1.7	62.8	48.6	113.2
10	9.6 ± 1.1	44	7.9 ± 0.8	29.9	32.7	92.8
12½	7.7 ± 0.8	32	6.0 ± 0.4	18.2	29.9	68.3
15	6.1 ± 0.6	24	4.9 ± 0.3	13.5	22.4	59.2
17½	5.6 ± 0.6	24	4.2 ± 0.1	8.2 (10.2)	24.0	31.7
20	5.0 ± 0.4	17.2	4.1 ± 0.1	7.8 (10.0)	17.5	30.0
25	4.8 ± 0.4	16.8	3.9 ± 0.1	7.0 (9.5)	17.3	26.5
30	4.8 ± 0.4	16.8	3.9 ± 0.1	7.0 (9.5)	17.5	26.5

M_{100} = mean values for the whole series of pupils.
M = mean values for the normal series.
M_7 = mean values for hemeralopes.
σ = standard deviation.
v = variation coefficient for the normal series M.

The figures in brackets denote values for the sum of the mean value and treble standard deviation computed by means of the average variation coefficient (47.9).

The threshold values are given in 1/1000 millifoot candles i.e. microfoot candles.

any means the most favourable with respect to vitamin A status but on the contrary the most unfavourable.

When the threshold values were being statistically treated it was noted that those for four of the pupils obtained after 20, 25 and 30 minute periods of adaptation exceeded the corresponding mean values by more than three times the standard deviation. Thus they had to be considered as irregular. Furthermore there were among the 96 remaining pupils 3 more whose threshold values obtained after 20, 25 and 30 minute periods of dark adaptation also exceeded the corresponding mean values of the new series by more than three

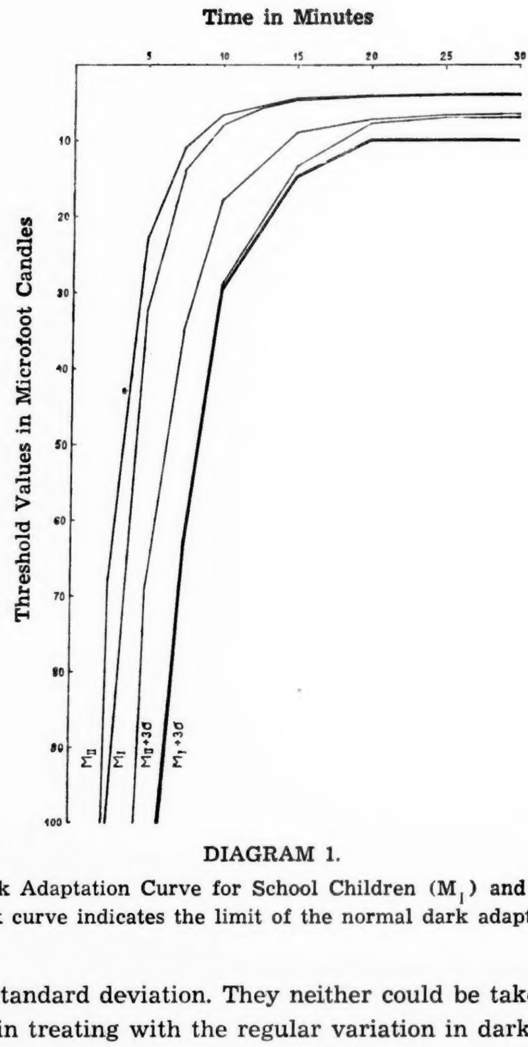


DIAGRAM 1.

Normal Dark Adaptation Curve for School Children (M_I) and Adults (M_{II}). The thick curve indicates the limit of the normal dark adaptation area.

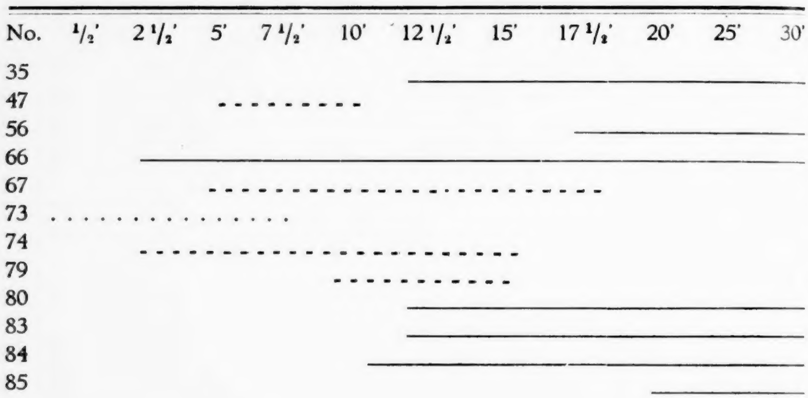
times the standard deviation. They neither could be taken into consideration in treating with the regular variation in dark adaptation.

Table 1 presents the averages of threshold values and the limits of normal distribution ($M+3\sigma$) both for the original series (M_{100}) and the new normal series (M) as well as averages for the group of pupils (M_7) whose dark adaptation appeared to be irregular.

Diagram 1 gives the normal curve of dark adaptation for children 7—14 years of age (M_I). Curve $M_I+3\sigma$ represents the limit between the normal and the subnormal region.

DIAGRAM 2.

The Divergence of the Irregular Dark Adaptation Curves from the Limit Curve $M_1+3\sigma$ at the Threshold Values Indicate by the Lines. ——————— hemeralopes, ------- border cases, ... deviation due to undefined irregularity in the functioning of the cones.



I have compared the threshold values of the members of the whole series to the limit curve and noted that the dark adaptation curve of 12 pupils deviated from the course of the limit curve as is shown in diagram 2. At the points marked with different kinds of lines the threshold values were greater than the corresponding threshold values of the limit curve $M_I+3\sigma$.

The second control group consisted of 50 persons, 15—50 years of age who were found to be healthy in routine examination and in most cases on the basis of a fairly long period of observation. I have judged the vitamin A status of this group as good on the basis of a dietary history, use of vitamin A over a fairly long period or the determination of the vitamin A concentration of the blood plasma. This group consisted of female prisoners from the Karvia Reserve Prison, who received 10,000 I.U. vitamin A daily until their dark adaptation improved, soldiers of the air flotilla stationed at Karvia, whose vitamin A status I considered good on the basis of their diet at the time of the tests, July—August 1940, members of the medical staff of the garrison hospital at Käkisalmi, and civilians from Kar-

TABLE 2.

Averages of Threshold Values of Normal Individuals 15—50 Years of Age with Ample Vitamin A Reserves.
M_{II} = mean values with their standard errors.
σ = standard deviation.

Dark Adaptation Period in Min.	M_{II}	σ	$M_{II} + 3\sigma$
½	346 ± 36	254	1108
2½	68 ± 7	49	215
5	23 ± 2.2	15	69
7½	11 ± 1.1	7.9	35
10	6.6 ± 0.57	3.9	18
12½	5.1 ± 0.33	2.2	12
15	4.4 ± 0.23	1.6	9.2
17½	4.1 ± 0.20	1.4	8.4
20	3.9 ± 0.17	1.1	7.3
25	3.7 ± 0.13	0.99	6.7
30	3.6 ± 0.13	0.96	6.5

via whose vitamin A status I considered good on the basis of dietary history and determination of the vitamin A concentration of the blood plasma.

Table 2 gives the average threshold values with their standard errors (M_{II}), the standard deviation (σ) and the limit values of the normal distribution ($M_{II} + 3\sigma$) for this group. Diagram 1 represents the average and limit values graphically.

Diagram 1 shows that the terminal parts of the two normal adaptation curves which were obtained in two different ways and one of which represents children's adaptation and the other chiefly adults' adaptation, almost completely unite. It cannot with certainty be explained why adaptation in the children's series took place more slowly in the beginning. If the delayed dark adaptation were the first sign of the deterioration of adaptation ability because of lowered vitamin A status, the divergence of the dark adaptation curves could be explained to be the result of the children's series' lower vitamin A standard. The children's group was examined in the

spring, and its vitamin status was not improved by administering vitamin doses prior to the dark adaptation tests, whereas group II was selected with respect to vitamin A status.

As, however, the average threshold values of the two normal series are congruent in the terminal parts of the dark adaptation curves (20—30 min.), which Nylund among others considers most important in estimating the dark adaptation of a subject, I have also in the middle phase of adaptation (10—17 $^1/_2$ min.) used the same limit curve between normal and subnormal adaptation for both children and adults, viz. the children's curve, whose threshold values are higher. Thus the range of normal variation in adults was slightly broadened in the phase of adaptation which has not been unanimously accepted for use as a criterion in estimating dark adaptation (Hecht and Mandelbaum '40, Dow and Steven '41, Yudkin *et al.* '43).

Since, on the other hand, in the biophotometer I used the smallest possible illumination quantity was 2.8 μfc., below which in all probability most of the subjects would have been able to fall in the final phase of dark adaptation, and since this fact most likely to some extent affected not only the averages of the threshold values but also the variation coefficients of the final phase of dark adaptation, I computed the 20—30 minute values of the terminal part of the standard limit curve according to the average variation coefficient however taking into consideration, for the aforesaid reasons, only the threshold values corresponding to 10—30 minute adaption periods in control group I.

Since the factors affecting the reaction rapidity of the physiological synthesis of visual purple are not definitely known, delay in dark adaptation cannot perhaps with complete certainty be asserted to arise from vitamin A deficiency, but must be considered a border case. According to test made by Tansley (l.c.) and Charpentier (l.c.) the regeneration of visual purple in animals with vitamin A deficiency was delayed but at the same time the total quantity of the visual purple regenerated in those animals was smaller than that in normal animals.

Deviation from the normal curve in the region of $^1/_2$—$7^1/_2$ minute periods (e.g. 73 in diagram 2, p. 20) has not been taken into

consideration because the seeing still at that phase is regarded to take place by means of the cones (Kohlrausch '22, Dieter '29, Willmer '46, etc.).

On the basis of the above I have decided upon the following rules for judging the dark adaptation of a subject when using a biophotometer:

1. Dark adaptation is normal, if the threshold values obtained after 10—30 minute periods of adaptation are smaller than or equal to the corresponding values of the limit curve $M_I + 3\sigma$, i.e. rounded off into whole microfoot candles:

≤ 30	μfc.	after	10	minutes
≤ 15	„	„	15	„
≤ 10	„	„	20	„
≤ 10	„	„	25	„
≤ 10	„	„	30	„

2. Night blindness is present, if the threshold values obtained after 20—30 minute adaptation periods are greater than the corresponding values of the limit curve, i.e.

< 10 μfc. after 20—30 minutes,

independently of what threshold values have been obtained after shorter adaptation periods.

3. Border cases are those retardations in dark adaptation in which one or more threshold values obtained after 10—15 minute adaptation periods are greater than the corresponding values of the limit curve, but in which the 20—30 minute values are normal.

Somewhat more subnormal cases are found from among the same material by the method used by me than by the original method of Jeans *et al.* This is due to the fact that a 10 minute dark adaptation period is not sufficient to give a complete picture of the dark adaptation ability.

Diagram 3 represents a very slight case of hemeralopia, but this case judged according to Jeans *et al.* would still be within the limits of normal. And as a matter of fact, the deviation from the

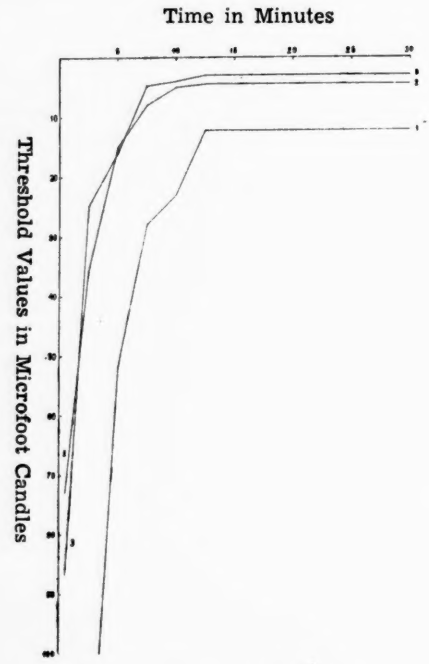


DIAGRAM 3.

Dark Adaptation of a Female Prisoner 40 Years of Age.
Curve 1 on May 23th, 1945. Lachrymation when reading.
Administering of vitamin A begun (10,000 I.U. daily).
Curve 2 on June 10th, 1945. Lachrymation has stopped.
Curve 3 on June 17th, 1945.

limit curve is slight. Nevertheless, the patient lachrymated when reading, and the lachrymation stopped when cod-liver oil was administered. Simultaneously night vision improved considerably.

Diagram 4 represents a more severe case of hemeralopia, which was also cured by cod-liver oil. When administering it was interrupted, the dark adaptation ability weakened perceptibly within eleven days but remained within the limits of normal for another week.

Diagram 5 presents two dark adaptation curves, the threshold values in the initial part of which are normal according to Jeans *et al.*, but the terminal part of which indicates that the dark adap-

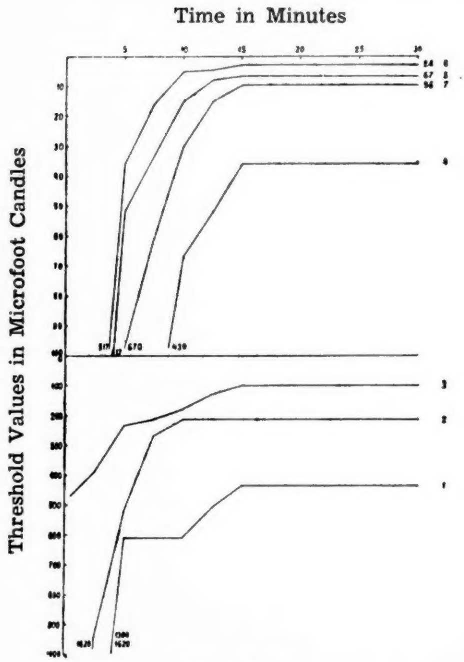


DIAGRAM 4.

Dark Adaptation of a Female Prisoner 27 Years of Age. Lachrymation and fatigue of eyes when reading. Received vitamin A 10,000 I.U. daily from April 19th to June 3rd, 1945. May 27th, 1945 the patient informed that she can read fairly long without her eyes tiring.

Curve 1 on April 19th, 1945.
„ 2 on May 6th, 1945.
„ 3 on May 13th, 1945.
„ 4 on May 20th, 1945.
„ 5 on May 27th, 1945.
„ 6 on june 3rd, 1945.
„ 7 on june 14th, 1945.

tation stopped after 10—15 minutes and the threshold values remained increased. The hemeralopia in these cases was relatively slight. In more severe hemeralopia (diagram 4 and table 5, p. 32) threshold values are more strongly increased, but the shape of the dark adaptation curve with its horizontal terminal part is similar to that in less severe cases. Hess ('09), Birnbacher ('24), Lindqvist ('38) and others consider this shape of the dark

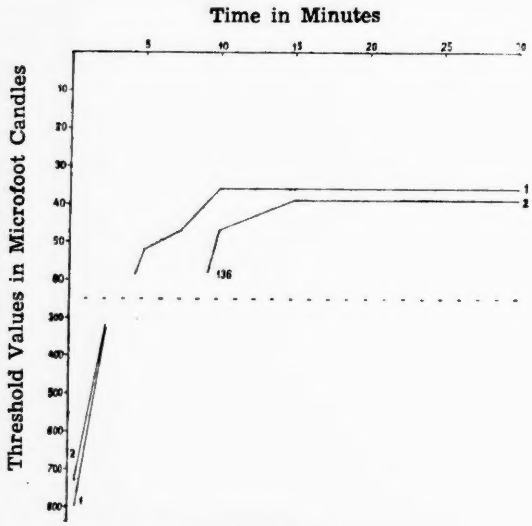


DIAGRAM 5.

Curve 1. Dark Adaptation of a Female Prisoner 35 Years of Age, April 19th, 1945.

Curve 2. Labourer 39 Years of Age, on December 11th, 1945. Fatigue, dyspnea, cough. A 10 mm wide line of epidermization of the mucosa of the lips. He had received only the card ration of butter. Roentgenoscopy: Pulm: 0, Cor: 0.

adaptation curve typical of hemeralopia. But there are cases of hemeralopia in which the shape of the curve is almost normal but the threshold values increased. Frequently these cases are not at all severe and judged according to J e a n s *et al.* normal.

In addition to these two types of curves there are relatively often cases in which the deviation in the initial part of the curve is particularly distinct. The threshold values of the terminal part of the curve are most frequently increased but may also attain a normal level. Provisionally I consider the latter as border cases. Marked retardation immediately at the beginning of dark adaptation occurs particularly in persons who have subjective symptoms also from light adaptation disturbances, such as dazzling of eyes in bright light. In such cases a bend in the initial part of the curve frequently occurs, which has been explained to correspond to the transfer from cone vision to rod vision (K o h l r a u s c h, D i e t e r,

Hecht, Willmer, l.c.). This bend appears to occur in hemeralopia cases already in the 3 minute light adaptation period more commonly than in normal cases (Cf. diagram 4). The increased threshold values already at the beginning of dark adaptation point to the fact that vitamin A has an effect also on the function of the cones.

The determinations of the vitamin A concentration of the blood plasma in this work were made according to the method currently used in the Department of Medical Chemistry, University of Helsinki, which method is based on van Eekelen's and Emmerie's method ('35) and which Pitkänen ('44) has described in detail in his dissertation on pages 54—57.

In this work I have chiefly estimated the subjects' vitamin A status and the occurrence frequency of hypovitaminosis-A in selected population groups only by means of dark adaptation tests, because the conditions under which I have done my work would have set insurmountable obstacles to making determinations of the vitamin A concentration of the blood plasma.

3. *Dark Adaptation Tests on Prisoners.*

The investigations have been made in the Reserve Prison at Karvia which is situated in northern Satakunta, about 120 kilometers to the north from the town of Pori. The Karvia Reserve Prison is a so-called open prison in which at the time of investigation mainly prisoners suitable for heavy outdoor work were kept. The work was strenuous physical labour in open air such as clearing of new land, ditching, hoeing of marsh land, logging, partly also farming and cattle-breeding.

During the time I acted as physician of the Reserve Prison my attention was drawn to the eye symptoms which were comparatively general with the prisoners and the etiology of which seemed unclear. Very many of the prisoners complained that when reading, especially in dim light, their eyes began watering and stinging and soon grew tired, in about half an hour, to such a degree that the reading became impossible. Many complained that bright light dazzled their eyes. Similar eye troubles were complained by prisoners in the Central Prison in Helsinki in the year 1939: among others working in the printing office of the prison became temporarily difficult for the prisoners due to the fact that the white paper surfaces blended their eyes. The prisoners told that before their arrival in the prison their eyes had been sound and the sight good, and that the disturbances in vision had been brought forth in the prison. Some of the prisoners had paid attention to the fact that when they were transferred to the so-called "probation class" and they were allowed to buy with their share of the wages extra milk among others, the eyes had become so much better that they were able to return to the work in the printing office.

As thus on the basis of the symptoms could be assumed that there existed a possibility of nutritional disturbances in the prisoners, I turned to the chief of the Department of Medical Chemistry of the University of Helsinki, Professor P .E. S i m o l a requesting him to give his opinion of the diet of the prisoners and the reasons of the symptoms noted. Professor S i m o l a advised that investigations would be made with regard to the vitamin condition of the prisoners, especially with regard to vitamin A. After having received permission from Dr. E. J. H o r e l l i, then the chief physician of the Administra-

TABLE 3.

Results from Dark Adaptation Tests on Prisoners in the Years 1939, 1941 and 1945 Grouped according to the Norms of Saksela (II) as well as Those of the Author (I).

Place of Investigation	Prisoners	Time of Investigation, Month and Year	Age in Average	Number	Adaptation Results											
					I						II					
					Normal		Border cases		Subnormal		Normal		Border cases		Subnormal	
					Number	%	Number	%	Number	%	Number	%	Number	%	Number	%
Helsinki Central Prison	Male	VI/'39		11	—	—	—	—	—	—	3	27.2	2	18.3	6	54.5
Karvia Reserve Prison	„	VI-IX/'39	33.6	118	—	—	—	—	—	—	24	20.3	6	5.1	88	74.6
„	„	II-III/'41	30.1	134	62	46.3	17	12.7	55	41.0	67	50.0	23	17.2	44	32.8
„	Female	IV-VI/'45	32.4	62	24	38.7	2	3.2	36	58.1	33	53.2	7	11.3	22	35.5
Total or Average		1939—45	31.9	325	86	43.9	19	9.7	91	46.4	127	39.1	38	11.7	160	49.2

tive Office of Public Prisons, I made in the years 1939, 1941 and 1945 dark adaptation measurements and partly also vitamin A and C determinations on prisoners.

In the summer 1939 I made adaptation investigations on 129 prisoners using the original method of Jeans and others and grouped the results as normal and subnormal according to the estimation norms presented by Saksela (l.c.). In the years 1941 and 1945 I used a slightly changed method and another manner of estimation which with their grounds have been introduced in Chapter 2. The results have been combined in table 3.

As could be expected on the basis of the eye troubles complained by the prisoners, the ability of dark adaptation was subnormal in very many prisoners. Of the male prisoners investigated in the summer 1939 nearly 75 % had a subnormal night vision, in early spring of the year 1941 the night vision was subnormal in 41 % (32.8 %), normal in 46.3 % (50 %) and uncertain in 12.7 % (17.2 %) of the cases. Of the 62 female prisoners investigated in the spring 1945, 58.1 % (35.5 %) had subnormal dark adaptation, 38.7 % (53.2 %) normal and 3.2 % (11.3 %) uncertain adaptation. Figures in the brackets stand for values reckoned according to the norms presented by Saksela.

The Effect of Cod-Liver Oil Concentrate on the Night Vision

In order to find out how the administration of vitamin A would influence upon this hemeralopia fairly generally appearing in Karvia Reserve Prison, I have experimented with vitamin A treatment in eight cases. It is to be regretted that pure vitamin A product was not available, but cod-liver oil concentrate had to be used, which contained 10,000 I.U. of vitamin A and 5,000 I.U. of vitamin D_2 per ml. 10,000 I.U. of vitamin A was daily administered orally over several weeks, and the ability of dark adaptation of the patients was investigated with intervals of one week. The investigation took place during the period of time from April 19th to June 21st, 1945. The persons investigated were female prisoners in the age between 22 and 41 years. The dark adaptation which was estimated according to the norms given previously in this work was at the start of the experiment subnormal in all of the investigated, in one case so slightly,

TABLE 4.

The Effect of the Use of Vitamin A on the Threshold Values and Eye Symptoms in Cases of Hemeralopia. 10,000 I.U. of vitamin A daily (+5,000 I.U. of vitamin D_2).

Age in Years	Threshold Values in Complete Adaptation		Administr. Period (weeks)	Eye Symptoms according to the Patient's Information		
	Before Administr. of Vitamin A	After Administr. of Vitamin A		Healing began: Threshold Value	Healing began: Time since Beginning of Treatment (weeks)	Symptoms
35	517	3.9	7	10	5	Tiring of eyes and mixing of letters when reading
27	430	2.8	5	6.7	4	
38	430	9.6	7	9.6	7	
26	149	23	3	23	3	Lachrymation and stinging sensation when reading. Administration of vitamin A interrupted after three weeks
22	80	15	3	19	1	
29	23	3.9	2	6	1	Lachrymation and stinging sensation
41	23	2.8	3	9.6	2	Mixing of letters when reading
40	12	2.8	3	4.3	1	Lachrymation when reading
32.3	208	8.0	4	11.0	3	In average

TABLE 5.

The Effect of the Use of Vitamin A on the Dark Adaptation of a 35 Years Old Hemeralope Female who Daily Took 10,000 I.U. of Vitamin A Since April 30th, 1945.

Dark Adaptation Period in Min.	Date in the Year 1945							
	19/4	6/5	13/5	20/5	27/5	3/6*)	11/6	21/6
1/2	1930	1770	800	430	360	328	211	252
2 1/2	1040	731	430	193	114	114	136	96
5	955	612	193	87	73	56	52	39
7 1/2	560	328	149	47	36	28	19	18
10	560	177	104	33	28	18	10	11
12 1/2	517	149	87	25	15	15	6	5
15	517	136	80	21	12	10	6	4
17 1/2	517	136	80	21	12	10	6	4
20—25	517	136	80	21	12	10	6	4

*) The patient informed that the vision began to be as before and that the letters did not "move" any more when reading.

that same according to Saksela's norms was to be considered as normal. None of the investigated was subjectively conscious of her night vision, but all had something to complain with regard to their eyes. All complained that when reading for about half an hour, the eyes began to water and that they burned like after long waking. Some told that when reading the eyes grew tired in about half an hour and the letters got mixed in "mist" so that the reading became impossible. Besides these eye troubles in those to be investigated no sickliness could be observed. Only one had a somewhat increased blood pressure (165/105 mm. Hg.).

The results have been described in table 4. Cod-liver oil had a decreasing effect on the threshold values in all persons tested and removed the disturbances in vision which had occurred when reading. A slighter disturbance in dark adaptation was healed quicker, in about 1—2 weeks, while a stronger hemeralopia required up to 7 weeks for curing when the vitamin A dosis mentioned above was used. The results from the dark adaptation measurements on subjects in whom hemeralopia appeared as strongest, are presented in table 5. In the first measuring the threshold values were clearly increased from the beginning of the dark adaptation to the end of same, the sensibility to light reaching its maximum after 12 1/2

minutes. From the table can also be seen how the ability of dark adaptation gradually was improved to normal during the use of vitamin A. After five weeks counted from the beginning of the vitamin A treatment the patient informed that her vision began to be better and that her eyes allowed her to read for a longer time without getting overstrained. On this point the sensibility to light of the retina in a complete stadium of dark adaptation was 10 microfoot candles, which I have previously fixed as the upper limit for the adaptation of 20—30 minutes. But already after a vitamin treatment of three weeks the ability of dark adaptation was so much improved that same, estimated according to the norms of Jeans and others or those of Saksela, on the basis of threshold values of $^1/_2$—10 minutes, was normal. Now the sensibility to light of the retina in complete stadium of dark adaptation was only 21 microfoot candles which in accordance with my investigations presented before in Chapter 2, I consider to be subnormal in healthy persons under 50 years of age with good vitamin A standard. When vitamin A was continually administered, the ability of dark adaptation also improved considerably during four weeks, reaching finally a level which equals to the average dark adaptation ability of healthy persons with good vitamin A standards. Before the administration of vitamin A the threshold values in the beginning of the dark adaptation were nearly eight times and at the end about 130 times greater than the corresponding threshold values after the treatment. Rich intake of vitamin A seems to improve even rather weak normal threshold values and thus to increase the ability of the eye to see in dim light.

Besides vitamin A, the patients received daily during this vitamin treatment also 5,000 I.U. of vitamin D. However, I do not consider the improvement of the ability of dark adaptation as a result of vitamin D due to the fact that hemeralopia occurred in prisoners also in summer-time, although they were working in the open air and were tanned by sun.

That the so-called learning factor (Palmer and Blumberg '37, Baum and Mc Coord '40, Turpeinen '41) seems to have no part worth mentioning in these improvements of the threshold values, but that same must be considered as results brought about by vitamin A, is revealed from the fact that in two

cases in which there was an opportunity to investigate the ability of dark adaptation 1—2 weeks after the interruption of the administration of vitamin A, it could be established that the threshold values had again increased (see diagram 4 on page 25).

Vitamin A Content of the Prison Dietary

The daily dietary of a prisoner doing hard work contained in the winter of 1945 about 1,200 I.U. of vitamin A in milk and margarine which was vitaminized and about 1,000 I.U. of carotene in carrots, rutabaga (Swedish turnip) and peas. In summer-time the vitamin A quantity was about 1,500 I.U. per man and day, but the carotene content varied depending upon whether carrots and rutabaga were available. According to the results of the investigations on dark adaptation the minimum daily requirement of vitamin A of a human being must be considered to be somewhat larger than the amounts of vitamin A included in the Finnish prison dietary.

4. *Dark Adaptation Tests on Soldiers*

Supplying of provisions for a war-time army involves a service problem which in earlier wars, until the wars in the last decade, never could be solved satisfactorily. Nutritional disorders such as oedema, scurvy and night blindness have been comparatively general phenomena among the war troops, not only in besieged towns and other "motties", but also among the besiegers. Still during the first World War numerous disturbances in nutrition occurred especially in the troops at the eastern front on both warring parties (F e i g '17, H o e r s c h e l m a n n '17, A s c h o f f and K o c h '18, P f e i f f e r '18, etc.). Disturbances occurred also among the naval forces (M c C a n n '27).

The wellfare service of the Finnish troops in the numerous wars in which the Finnish people have been engaged earlier during the last thousand years, has been poor in general, but especially the medical service has been very defective due to the fact that before the year 1750 there were still no Finnish physicians nor other schooled medical staff which would have been able to arrange the medical service of the troops on a satisfactory standard. Still in the wars of 1788—90 and 1808—09 the medical service of the Finnish army — in spite of detailed planning — acted most unsatisfactorily due to the same reasons as previously, before all therefore that there was not enough of schooled medical staff (S o i n i n e n '42). Especially infectious diseases, such as recurrent fever which the very common and destructive "field disease" has later been considered to be caused immense destruction (S o i n i n e n). What the part of the nutritional disturbances have been in the great losses, is impossible to decide, because the cause of death has mostly not been duly established. But it is most likely that nutritional disorders by no means have been lacking in earlier Finnish wars, in consideration of the general providing possibilities.

Generally speaking: when a small nation even to-day gets involved in a war of a longer duration lasting for a longer time than the latency period of the deficiency diseases is reckoned to be, the threat of these diseases is existing owing to the critical deterioration of the provision supplies sooner or later.

As I had commenced the research concerning the vitamin condition of prisoners already in the summer of 1939 and had stated that the vitamin A standard of the prisoners was unsatisfactory, it was only natural that when the war broke out in the autumn 1939, I began to make observations with regard to the provision and vitamin condition of the unit into which I had been commanded. However, the war of 1939—40 even with the extraordinary reserve training could not noteworthy affect the supplying of provisions to the troops. Manifestations of deficiency diseases could not be found due to the short period of time the war lasted. S i m o l a's and S a k s e l a's dark adaptation investigations on soldiers in 1940 showed that he vitamin A content of the provisions used by the soldiers investigated, has been comparatively good. The decrease in the ability of dark adaptation was more rare among the soldiers than in other groups of people. The soldier material, it is true,

TABLE 6.

Results from Dark Adaptation Tests on Soldiers in the Years 1940—43 Grouped According to the Estimation Norms of Saksela (II) as well as Those of the Author (I).

Place of Investigation	Time of Investigation Month and Year	Number	Age in Average	Adaptation Results											
				I						II					
				Normal		Border cases		Subnormal		Normal		Border cases		Subnormal	
				Number	%	Number	%	Number	%	Number	%	Number	%	Number	%
Karvia	VII—VIII/'40	6	21.8	5	—	—	—	1	—	5	—	—	—	1	—
Luumäki	VI/'41	4	23.8	4	—	—	—	—	—	4	—	—	—	—	—
Käkisalmi	I—VI/'42	113	27.1	69	61.1	14	12.4	30	26.5	81	71.7	6	5.3	26	23.0
"	VII—XII/'42	81	24.5	60	74.1	6	7.4	15	18.5	69	85.2	—	—	12	14.8
"	I—VIII/'43	41	26.2	30	73.2	2	4.9	9	21.9	34	82.9	—	—	7	17.1
Total or Average		245	26.0	168	68.6	22	9.0	55	22.4	193	78.8	6	2.4	46	18.8

as to the condition of health was selected and in the best age, and the peace time previous to the war with regard to provision supplies had been in ascending line, due to which the vitamin A reserves of the soldiers had apparently been ample when they went to war.

The winter war, however, revealed how difficult it can be to procure provisions containing vitamin C, such as potatoes and rutabaga, for the frontline troops during severe frost. It is most probable that the vitamin C reserves of the soldiers decreased with good speed during the few months of the winter war when the nourishment of the frontline soldiers almost completely was lacking in potatoes. Frozen potatoes which certainly would have been available, the soldiers generally did not desire to use in preparing of their food. According to the investigations made by Simola ('41) freezing in itself does not decrease the vitamin C content of the potatoes. However, as far as is known, no certain clinical symptoms appeared owing to the comparatively brief deterioration of the vitamin C supply.

Actual experimental investigations on the dark adaptation ability of the soldiers were made by me mainly only during the last war in the years 1942—43. The material covered in all 245 soldiers including 10 whose dark adaptation had been investigated earlier. The results are given in table 6 estimated both according to my own principles and those presented by Saksela. Even in this test group I received distinctly more subnormal cases of dark adaptation when employing my own norms in the estimation of the results than when the estimation was made using the rules of Saksela.

Within the whole material there were 68.6 % with normal and 22.4 % with subnormal ability of dark adaptation whereto come 9 % of cases in which the dark adaptation must be considered to lie on the border-line. Estimated according to Saksela's method the figures were 78.8 %, 18.8 % and 2.4 % respectively. When these figures are compared with the dark adaptation results Simola and Saksela obtained during the previous war in investigations on front soldiers, when there were 87.5 % of normal, 9.6 % of subnormal and 2.9 % of border cases, a distinct deterioration can be observed in the soldiers' dark adaptation. The difference in my opinion has been still greater on the ground that the method used by

me when the dark adaptation grew worse, reveals more slighter cases of hemeralopia than the method employed by Saksela.

These results do well tally with the results from the investigations made by Simola in February—July 1942, according to which the average vitamin A content of the blood was distinctly lower than that in the investigations made in the years 1938—41.

When the results obtained in the former and latter halves of the year 1942 are compared with each other, it may be observed that the number of normal dark adaptation cases has considerably increased in the latter part of the year. This I consider to result from the improvement of the vitamin A condition which partly was due to the increase in the use of milk. In the summer 1942 to the environs of Käkisalmi moved a considerable number of civil people and brought cows with them. The soldiers entered into communication with the civil people and procured additional milk in spite of given orders. Many of the soldiers were originally natives of the environs of Käkisalmi, and thus when their relatives arrived back in their native place, they were in the opportunity of visiting them more often than previously.

In the first part of the year 1943 the number of subnormal dark adaptation cases increased somewhat, which can be considered as a seasonal variation and deriving from the decrease in the vitamin A content of milk.

5. *Dark Adaptation Tests on Adult Civil Persons*

With the new victories gained by nutritional research more than ten years ago, the question became urgent, in which degree the dietary of the people in different countries corresponds to the new discoveries of the science and in which degree its quality and composition possibly are causes of the phenomena of illnesses occurring in an obviously increasing degree simultaneously with the human development. The solving of these questions got new impetus when the Health Organization of the League of Nations included them in its most urgent program.

In Finland a committee appointed by the state, the Kansanravitsemuskomitea [The People's Nutrition Committee (PNC)] has made in the years 1936—40 an especially extensive and thorough investigation on the nutrition of the people. The PNC could state that in the groups of relatively poor people quantitative subnutrition was found in 21 % of the families investigated and that 4 % had a directly scant and miserable supply of proteins. In the spring 71 %, in the autumn only 42 % of the investigated families remained below the requirement value as to the supplies of energy, while 40 % of the families obtained less proteins than the requirement value accepted by the Health Organization of the League of Nations implied [Virtanen and Turpeinen[1]].

As to the vitamin condition of the Finnish people, the PNC stated that in the spring in 94 % of the investigated families the intake of vitamin A remained under the requirement value. During the summer, however, the vitamin condition improved into the effect that in the autumn "only" 60 % had the vitamin A intake insufficient. In the conclusions the PNC stated that "the lack in vitamin A is evidently the greatest problem of the nutritional question of our people and the improvement of same an important and urgent task" [Virtanen and Turpeinen[2]].

At about the very same time Simola planned a series of investigations which were aimed among others to solve experimentally the vitamin standards of the different groups of the Finnish people, the occurrence frequency of the deficiency states, the vitamin quantities included in the various food-stuffs in the dietary of the Finnish people and to find out means for the prevention of the deficiency diseases by developing the use of nourishment. Simola and his collaborators Saksela and Pitkänen have published the results of several investigations concerning these objects, partly in the form of academic dissertations.

The original purpose of the work at issue was at the side of the exposition of the vitamin condition of the prisoners also the investi-

[1]) Tutkimuksia kansanravitsemustilan parantamiseksi (Investigations aimed at the improvement of the nutritional condition of the people). Helsinki 1940, pages 64—126.

[2]) ibid. pages 81 and 88.

TABLE 7.

Dark Adaptation Tests on Adult Civil Persons Grouped according to the Norms Employed by Saksela (II) as well as Those of the Author (I).

Place of Investigation	Time of Investigation Month and Year	Number	Age in Average	Adaptation Results											
				I						II					
				Normal		Border cases		Subnormal		Normal		Border cases		Subnormal	
				Number	%	Number	%	Number	%	Number	%	Number	%	Number	%
Karvia	VIII—XII/'40 I—V/'41	53	30.4	40	75.5	—	—	13	24.5	47	88.7	2	3.8	4	7.5
Käkisalmi	I—XII/'42 I—VIII/'43	27	33.4	17	63.0	2	7.4	8	29.6	22	81.5	1	3.7	4	14.8
Total or Average		80	31.4	57	71.3	2	2.5	21	26.2	69	86.2	3	3.8	8	10.0

gation of the vitamin condition of the population living in the rural community of Karvia. Due to the intervening wars the original working plan must partly be changed and restricted. Because of this reason the investigation concerning the population group of Karvia community had to be reduced to much less than originally had been planned. The gathering of the soldier material during the war respectively limited the dimensions of the investigations which I had intended to make on the vitamin condition of the population group consisting of war emigrants in Karelia.

Owing to practical reasons the results of the investigations on children of school-age made in the community of Karvia have been treated separately in the following chapter.

The results of the dark adaptation tests on adults are given in table 7.

A total of 80 persons have been investigated, of which 53 were investigated at Karvia and 27 at Käkisalmi. Of the group belonging to the population of Karvia 75.5 % had a normal and 24,5 % a subnormal dark adaptation. Estimated according to Saksela there were 88.7 % of normal and 7.5 % of subnormal and 3.8 % of border cases. The lastmentioned figures, which have been obtained by the same method as the dark adaptation results of Saksela and Simola at Kemijärvi and Pori and those of Pitkänen in Helsinki, indicate that a normal ability of dark adaptation and therewith a normal supply of vitamin A would have been more common in the population of Karvia than that of Kemijärvi community and the towns Helsinki and Pori.

As I have used in the final estimation of the dark adaptation also the threshold values of the last part of the adaptation curve, the results received by me can also be compared with the results obtained when employing the method used by Nylund (l.c.). Nylund who has investigated dark adaptation also in various diseases, declares to have found a normal adaptation in 62.5 %, subnormal in 24.6 % and adaptation which is to be considered as a border case in 12.9 %. Compared with the results obtained by Nylund, in the population of Karvia the normal dark adaptation was distinctly more common than in the group of clinical cases of Nylund (309 persons), in which 31 cases of malignant tumours, 20 cases of thyreotoxicosis and 7 cases of cirrhosis hepatis were

included, in which according to literature, I refer to the references of literature made by Lindqvist, Saksela and Nylund, hemeralopia often occurs. My own material covers in addition to healthy persons, secondary states of war injuries, scabies, hemorrhoid, rheuma and gingivitis cases and one case of hepatitis acuta of a comparatively slight character. In the cases of Nylund, hemeralopia was not characteristic of the hepatitis, at least not in the beginning of the disease, while Saksela again in serious cases of hepatitis regularly found low values of vitamin A and carotene in the serum and in 3 cases out of 4 a subnormal dark adaptation.

When the results of the dark adaptation of adults is compared with the dark adaptation results of children of school-age, which are demonstrated in table 9, page 45, it can be noted that in children the normal dark adaptation was more common than in adults among the population of Karvia.

The group of population of Karvia includes 12 war emigrants from Hiitola, whose dark adaptation was normal except in one case, viz. the above mentioned case of hepatitis acuta in which the dark adaptation ability according to my estimation was slightly disturbed, but normal according to Saksela's norms. In another case, a war emigrant of 56 years who had diphyllobothriasis and keratitis phlyctenulosa o. dx., the threshold values of the adaptation time of 10—30 minutes were still within the range of normal, but the threshold values of $1/2$ and 2 $1/2$ minute periods border values according to Saksela. The vitamin A content of the serum was 134 I.U. per 100 ml.

When comparing the group of population living at Karvia with the group of emigrants who had returned to Käkisalmi, a distinct decrease in the number of normal dark adaptation cases can be observed (63 %) which presumably is owing to the obviously more deficient nutritional conditions of the emigrants who had returned to Karelia. The number of the cases, however, is altogether too small for drawing any conclusions with regard to the vitamin A standard of the people who had returned to their native places. Yet, it is to be noted that among the emigrants and soldiers in the same place the difference in frequency of the normal results of dark adaptation is very slight (corresponding figures 67.7 and 63 %).

6. *Dark Adaptation Tests on School Children*

Children have been objects for a special interest of the vitamin investigators nearest because of the fact that according to experimental investigations a growing organism in general seems to be more liable to get symptoms of vitamin deficiencies than a fully developed body, and that the differential diagnostic difficulties of the deficiency diseases with regard to other, especially with regard to chronic syndromes, are not as great in children as in adults.

As the children of school-age form an especially suitable material for more extensive serial investigations and as in the dark adaptation tests founded on subjective observations younger children hardly can be used, extensive investigations have been made in foreign countries as well as in Finland on school children in order to state the generality of the hypovitaminosis-A. As to the foreign literature, reference is made to the extensive literature references of both Saksela and Nylund. The results of the investigations made in Finland I have compiled in table No. 8. The investigations have been made in the years 1938—1941. A normal dark adaptation which with a rather great certainty is an evidence of a sufficient vitamin A standard (Nylund '41) occurs varyingly in different places and according to different investigators in 38.8—95 % of the investigated school children, the number of which is 1738. A subnormal dark adaptation has been stated in 0—36.7 % (in average in 21.4 %). The rest have been uncertain border cases. The difference between the occurrence frequency of the hypovitaminosis-A stated by Simola and Saksela on one side and Nylund on the other, is obviously due to difference in methods, as one part of Simola's and Saksela's material otherwise is comparable with Nylund's material, but the occurrence frequency of the hypovitaminosis-A in the early spring of 1939 among the primary school children of Helsinki was according to Nylund considerably greater than according to the investigations of Simola and Saksela.

Simola and Saksela stated a distinct deterioration in the vitamin A condition in the early spring compared with the investigations made in previous autumn. In the groups of primary school children investigated by Simola and Saksela, Leppo and Nylund there were in average 58.5 % of normal, 25.7 % of subnormal and 15.8 % of border cases.

These figures are well in accordance with the results given by the investigation of the Kansanravitsemuskomitea[1]) made in the year 1936 on the nutrition of the school children. According to these investigations a dietary of the so-called scant food types was used in the province of Uudenmaan lääni by 23.7 %, in the province of Oulun lääni by 42.0 % and in the province of Viipurin lääni by 43.3 % and in the whole country by 34.3 % of the primary school children. The dietary of these contained very little of butter and whole

[1]) Tutkimuksia kansanravitsemustilan parantamiseksi (Investigations aimed at the improvement of the Nutrition of the People.) Helsinki 1940, page 146.

TABLE 8.

The Occurrence of **Hypovitaminosis-A** in Finnish School Children in the Light of Dark Adaptation Tests.

Investigator	Method	Number of Children Investigated	Age	Adaptation Results						Time and Place of Investigation
				Normal		Border Cases		Subnormal		
				Number	%	Number	%	Number	%	
Simola and Saksela	Biophotom.	203	10.5	135	66.5	31	15.3	37	18.2	X-XII/'38 Helsinki
,,	,,	311	10.5	181	58.2	46	14.8	84	27.0	III-V/'39 Helsinki
,,	,,	150	10.3	102	68.0	13	8.7	35	23.3	III-V/'39 Kemijärvi
Leppo	,,	100		41	41	24	24	35	35	V/'38 Viipuri
Nylund	Nylund	98	9—15	38	38.8	24	24.5	36	36.7	II-IV/'39 Helsinki
,,	,,	20	9—15	19	95	1	5	0	0	II/'40 Environs of Helsinki
Turpeinen	,,	856	7—13		(82.9)			(146)	17.1	Spring '41 Different Places
		882		516	58.5	139	15.8	227	25.7	
		1738						373	21.4	

milk and otherwise was monotonous, containing mainly bread, potatoes, skim-milk, salted fish, sugar and coffee, so that the content of the fat-soluble vitamins was minimal.

When these results of investigations obtained in different places and by employing different methods are compared with each other, it must be considered as in a way natural result phenomenon that of the children of poor people only about 40—70 % had a normal vision in dim light and a normal vitamin A standard and that 30—60 % at least temporarily were suffering of a latent deficiency disease, hypovitaminosis-A.

My own material which includes 100 school children of the primary school in the church village of Karvia parish has already been treated for the most part in connection with the methods. The estimation of the dark adaptation ability has been made by employing the statistical method according to which also Turpeinen ('42) has treated his material. The results of the dark adaptation measurements on school children are given in table 9.

TABLE 9.

The Occurrence of Hypovitaminosis-A in the Pupils of the Primary School in the Church Village of Karvia in March—April 1941 in the Light of Investigations on Dark Adaptation. The figures in brackets have been obtained when estimating the material according to Saksela's rules.

	Number	Age in Average	Adaptation Results		
			Normal %	Border cases %	Subnormal %
School Children	100	10,6	89 (93)	4 (4)	7 (3)

On the basis of the adaptation tests can be stated that the vitamin A standard in the pupils of the primary school in the church village of Karvia was in the spring 1941 considerably better than elsewhere in Finland where investigations have been made coincidentally, excluding the town of Helsinki where according to Turpeinen's investigations the hypovitaminosis-A occurred only in 1.7 % of the investigated. The reason for the relatively good vitamin A standard in the primary school children of the church village of Karvia may without difficulty be found in the fact that in Karvia parish dairy

products were produced in excess, every household having one cow or more, and there are in the parish only few people living on card rations in the present time of restrictions. To the school district of the church village belong families with an average living standard. It is also possible that the results have been somewhat influenced by the fact that the comprehension of the inhabitants of the parish seems to have gradually changed in some degree as to the importance which the care and proper nourishing of the children have for their health. It is also reflected in the fact that cod-liver oil as a roborant for children has gained more appreciation. Still a little more than ten years ago when I commenced my activity as physician at Karvia community, it was rather hopeless to recommend cod-liver oil or to prescribe it or its concentrates even in medicine mixtures, for such medicines could easily be left at the apothecary's.

The investigations described above on dark adaptation and the common occurrence of the rickets in Finland clearly show how necessary it would be to provide information and advice and do propaganda to explain to the people how common the deficiency diseases are and to avert the danger caused by them to children. As far as deficiency diseases, especially among children are concerned the importance of preventive measures cannot be overemphasized in the interests of public health.

PART II

STUDIES ON VITAMIN C

CHAPTER III

ON THE ESTIMATION OF THE NORMAL VITAMIN C STANDARD OF MAN

As it is known, the ability to form vitamin C in the body is found to be lacking only in human beings, monkeys (Hart and Lessing '13) and guinea-pig (Holst and Frölich '07), and according to some authors, to a certain extent at least, in red deer (Wiedemann '38), calf (Hjärre and Lilleengen '36) and pig (Plimmer '20). All other animals possess the faculty of synthesizing it and do not fall ill with scurvy if they do not get any supplies of vitamin C from outside. Therefore, vitamin C in such animals who produce it themselves is no vitamin in a proper sense of the word, but should rather be regarded as a hormone. That the animal organism does indeed possess the faculty of a spontaneous formation of vitamin C, was proved by the circumstance that scurvy in guinea-pigs could be improved by administering to them organs of rats kept on a diet deficient in vitamin C (Parsons '24, etc.). Giroud *et al.* ('36) believe that also animals susceptible for scurvy, as well as man possess an ability to synthesize vitamin C, which, however, is insufficient to prevent scurvy.

Phylogenetically taking it is to be assumed that the remote ancestors of man were able to manage without an outside supply of vitamin C. It is hardly possible to find out now why and at what particular stage of the phylogenetic development the capability to synthesize vitamin C was lost — this loss being one of the most fatal to man, fatal in respect that he would have needed it badly during many a phase of his later migrations. It can be only supposed that this happened before the genus branched off to ape and man. Von Wendt and Müller-Lenhartz ('39) assumed that atrophy of the cells, which formed vitamin C, was due to the circumstance that such a formation had become unnecessary owing to the exceedingly high content of vitamin C in food.

Rohmer and Bezssonoff ('42) maintain that the human infant is able to synthesize vitamin C up to the age of 12 months. Several authors

do not consider it established that an adult should not also be able to synthesize vitamin C and in that way satisfy part of his need [Giroud *et al.* '36, Rietschel and Mensching '39, Bicknell and Prescott '47), Simola (private communication)]. Ingalls *et al.* ('38) found that a nursing mother, although being daily administered less than 20 mg. of vitamin C in her food, still produces daily quantities of milk containing considerably more vitamin C; therefore, either the vitamin C reserves of maternal tissues have been used, or a synthesis of vitamin C has perhaps occurred. Although one and a half to four times stronger concentrations of vitamin C have been found in the umbilical blood of the newborn than in the maternal blood (Braestrup '39, Lund and Kimble '43), yet it has been proved on the other hand that the vitamin C concentration in the blood plasma of the young infant depends on the amounts of vitamin C supplied in the food after the quantities of it receaved from the maternal organism have been used up, which occurs approximately at the age of one month (Mindlin and Ingalls '39). The average vitamin C concentration in the blood plasma of the newborn infant is according to Braestrup 0.69 mg%, but already two weeks after birth it can be only 0.1 mg% (Mindlin '38). An administration of 20 mg. ascorbic acid per day increased the vitamin C content of the plasma to 0.4—0.8 mg% according to Mindlin ('38) and to 0.56—0.76 mg% according to Braestrup ('39).

Observations by Levine *et al.* ('41) on alkaptonuria in premature infants which were cured by administering ascorbic acid, seem to indicate that there is no synthesis of vitamin C towards the end of the fetal stage. Since the requirement of this vitamin is relatively greater during the years of growth than in adults, it does not seem to serve the purpose that the ability to form vitamin C should develop gradually during the time of life. In fact, the content of vitamin C in the plasma of adult individuals decreased in the course of 41 to 129 days to the zero level, if this vitamin was completely absent from the diet (Rietschel and Mensching '39, Rietschel and Schick '39, Crandon and Lund '40, etc.).

The vitamin C concentration of human milk also varies most sensitively from 1 to 10 mg% in accordance with the vitamin C content in the diet of the mother (Bicknell and Prescott, l.c.). Furthermore, Crandon experimentally induced in himself, through a diet fully deficient in vitamin C, within a shorter time than 6 months, a prevented collagen formation in the scar tissue of a wound, which is characteristic of scurvy, and he proved also that synthetic ascorbic acid rapidly brought about the formation of collagen and a complete healing of the wound. Tobler's ('18) observations indicate that children indisputably develop scurvy, provided the diet is deficient in vitamin C for a sufficient length of time. In Tobler's series the latency of scurvy lasted 8 to 12 months. Göthlin ('37) and Fox *et al.* ('40) proved that partial deficiency in vitamin C produced in man scorbutic changes which improved or were arrested by administrations of vitamin C. Experiments on monkeys (Hart and Lessing '13, Fraser '42, Shaw, Phillips and Elvehjem '45, etc.) and numerous observations on human individuals

proved that a lack of the capability to synthesize vitamin C is common to all primates, and if their diet is wholly or partly deficient in this substance, they develop manifest scurvy in the course of 6 to 12 months, part of which is to be regarded as a latent period (Tobler '18, Fox *et al.* '40, Grandon *et al.* '40).

The tissues of all animals, also of those capable to synthesize vitamin C, seem to react by scorbutic changes to a lack of it, provided this deficiency condition can be brought about in their organism. Jonsson *et al.* ('42) demonstrated that parallel with the progress of avitaminosis-A in rats the vitamin C content also diminished in the organs and plasma, and simultaneously with eye symptoms the teeth also underwent changes resembling those associated with chronic scurvy.

The liver being most closely associated with the metabolism of vitamin A, the idea is suggested that the synthesis of vitamin C occurs in the liver. (Stepp, Kühnau and Schroeder: Die Vitamine, 6th edition, page 277). Pfiffner *et al.* ('35) demonstrated that the synthesis of vitamin C in dogs does not take place in the adrenal glands. According to the experiments of Sutton, Kaeser and Hansard ('42), the formation of vitamin C does not take place in either adrenals, ovaries or pituitary of the rat. Sutton *et al.* are inclined to assume that "the synthesis of ascorbic acid is not a specific function of any single gland or tissue but more probably a general metabolic function". Ascorbic acid may be considered to belong in most animals to the group of so-called tissue hormones.

From the site of formation, wherever it may be, in animals synthesizing vitamin C and from the chyle through the wall of the intestine in animals incapable of this synthesis as well as in man, — vitamin C is carried by the blood, dissolved in the plasma, to all the organs of the body in which it is found, however, in different concentrations.

On table 10 I have collected the figures reported by different authors, representing concentrations of vitamin C in organs, tissues and secretions both in animals synthesizing vitamin C and in those incapable of this synthesis as well as in man. The table illustrates that the adrenal gland is the animal organ richest in vitamin C. An increased function of the organ raises the level of its vitamin C concentration (Giroud '36, Tonutti ('39). The greatest requirement of vitamin C have those active cells which form some cell-products, such as intercellular binding substance, hormones, ferments, antitoxins or pigments. The richness in vitamin C of an organ is not an evidence of the storage but of the actual requirement at the moment (Tonutti '39, Scheunert '40). Several authors (Euler and Malmberg '35, Fox and Levy '36, Broch '39) have shown that administrations of massive doses of vitamin C do not produce a storage greater than normal in the organs of test animals. Fox and Levy, Broch, etc. have also proved that in the guinea-pig the adrenal glands and other organs as well react, where their vitamin C concentrations are concerned, with great rapidity to a lack of this substance. In the course of 10 days, during which slight scorbutic changes occur in the teeth, the concentration of vitamin C in the adrenal glands di-

TABLE 10.

Ascorbic Acid Content in mg% in Blood Plasma, Adrenal Gland, Liver and Milk of Man and Some Animals.

	Blood plasma		Adrenal Gland	Liver	Milk
	Range	Average			
Man:	a)	b)	c)	d)	e)
adult[1])	0.0—2.5		15	7	1—10
infant[2])	0.28—1.28	0.69	48	21	
Guinea-pig[3])	0.82—1.20 0.87—1.73		111—214	45—52	29
Rat[4])	0.82—1.80	1.18	340—368	25	0.4
Dog[5])	0.55—0.94				
Horse[6])	1.14—1.74	1.35 0.54 *	123	18	8.65—19.7
Cow[7])	0.79—2.64	1.32	148	19	ca. 2.0
Sheep[8])	0.43—0.82				0.80
Goat[9])	0.6—0.8				0.5—2.0
Frog[10])	0.7—0.9			18	

1a) Abt *et al.* ('36), Trier ('38) Dagulf ('39)
1cd), 2cd) Giroud *et al.* ('37)
1e) Bicknell and Prescott ('47)
2ab) Braestrup ('39)
3) v. Eekelen and Kooy ('34), Fox and Levy ('36), Broch ('39), Ecker and Pillemer ('40), Frommel and Goldféder ('46), Houston and Kon ('39)
4) Sure *et al.* ('39), Jonsson *et al.* ('42)
5) Ralli and Sherry ('40)
6a) Radeff ('41), b)* Westermarck
6cd, 7cd) Natscheff and Georgieff ('40), 6e) Cimmino ('40)
7) Radeff ('41)
7e) Kon and Watson ('37), v. Wendt, Lojander and Ehrström ('38), Lojander ('39), Holmes *et al.* ('43)
8) Satterfield *et al.* ('42)
9) Richmond *et al.* ('40)
10) Sievers ('39)

minishes to about one tenth of the normal or to 12—20 mg%, and in the liver to 2.6 mg%. In a fully developed scurvy the amount of vitamin C in the adrenals is below 2.4 mg% (Fox and Levy). If the guinea-pig is administered daily unequal doses of ascorbic acid smaller than normal, the observation is made that blood plasma, adrenals and other organs contain correspondingly smaller amounts of ascorbic acid, as illustrated by table 11 compiled according to Broch ('39) and Ecker and Pillemer ('40) and Giroud *et al.* ('35).

It therefore appears evident that the vitamin C standard of the guinea-pig, where the amount of this substance is maximal in plasma, the adrenals and

TABLE 11.

Ascorbic Acid Content in the Adrenal Glands and Blood Plasma of Guinea-pigs when Different Daily Doses of Ascorbic Acid Are Administered (According to Broch (l.c.) and Ecker and Pillemer (l.c.) and Giroud *et al.* '35).

Daily Dose of Ascorbic Acid mg.	Adrenal Glands mg%	Blood Plasma mg%	
		Range	Average
0	1.20*	0.0—0.05	0.02
0.5	19	0.0—0.21	0.09
1.0		0.05—0.36	0.13
2.0	24	0.12—0.54	0.33
5.0	44	0.39—0.74	0.57
10.0	69	0.83—1.44	1.03
20.0	90	0.87—1.73	1.08
100.0	109		
Cabbage and root vegetables	111		

* Own result

other organs, and which remains undiminished only provided the animal receives a natural food, or such as corresponds to it also with regard to the vitamin C quantities, represents the normal and optimal standard of this substance in the guinea-pig. Its very high concentration in the milk of this animal, on an average 29 mg% (Houston and Kon '39), gives reason to suppose that the need of vitamin C in the guinea-pig is great ever since its birth. As illustrated by the table, the concentrations of vitamin C in the organs and plasma of animals capable of synthesizing it are approximately consistent with the figures for guinea-pig, when this animal is on a natural diet. Large-sized animals, such as horses and cows, also have a comparatively high concentration of vitamin C in the blood plasma, i.e. 1.14—1.74 mg% and 0.79—2.64 mg% respectively; these concentrations must be regarded as optimal for these animals and their cell-functions, since in them the synthesis of vitamin C is evidently regulated by the actual requirement of the cell-life in organs and tissues. Sheahan ('47), however, has stated noticeably lower plasma ascorbic acid values in certain domestic animals during winter months, in cows ranging from 0.14 to 0.92 mg%, the average being 0.38 mg%. The plasma ascorbic acid values had a tendency to decrease during the winter months and to increase in the spring.

It therefore appears that all the animals examined hitherto, even frogs (Sievers '39), possess a standard of vitamin C which corresponds to that of a guinea-pig living on a natural diet. It can therefore be designated as the

normal vitamin C standard in animals. The vitamin C concentration in the blood plasma, which amounts to about 0.6—1.0 mg%, is one of the characteristics of this standard.

On Characteristics in the Normal Vitamin C Standard of Man

Considerable endeavours were made in the course of the last decade to elucidate the features characteristic of the normal vitamin C standard in the human organism, but without complete success. It was proved by numerous tests that man is also unable to store vitamin C in his organism over and above certain maximum quantities — thus evidencing his similarity in this respect with the animal metabolism in vitamin C — but the human organism eliminates surplus ascorbic acid through the kidneys. The standard in which the greatest part (50—80 %) of the quantity of vitamin C ingested is excreted through the kidney, is called the state of saturation. If on the other hand the organism is not saturated, it is said to have a certain saturation deficit. Its extent is marked by the amount of vitamin C necessary to produce in the organism a state of saturation. Attempts have been made to assess the vitamin C standard by estimating this deficit or by means of the saturation test. On the adoption of this method of research, a complete saturation with vitamin C was at first considered normal and the saturation deficit regarded as hypovitaminosis-C. Gradually, however, the limits of the normal condition were extended and smaller vitamin C deficits, even up to 2,000 mg, which were found to occur also in subjectively and clinically healthy individuals were called physiological saturation deficit (Baumann '37).

The saturation test is, however, a fairly time-consuming method, nor does it seem to yield fully comparable results. Therefore determinations of vitamin C in the blood plasma or serum are used more and more frequently, especially for serial tests; such estimations are regarded by Stepp, Kühnau and Schroeder (Die Vitamine, page 284) as the best means of studying the standard of vitamin C. Uncertainty and differing views have arisen as to what values are to be considered normal and what subnormal. The estimation of the results is also partly dependent on the method which has been used for the determination of the vitamin C concentration of the blood. Abt and Farmer ('38) have estimated the results obtained by their own method as follows:

over 0.70 mg%	=	normal
0.50 — 0.70 „	=	subnormal or at least suboptimal
below 0.50 „	=	scorbutic

The results obtained by the method of Farmer and Abt are estimated in a somewhat similar way also by Wortis, Liebmann and Wortis ('38) as well as Greenberg, Rinehart and Phatak ('36). 0.7 to 0.9 mg% were adequate but not optimal. Baumann (l.c.), as well as Gaehtgens and Schwan ('41) consider values below 0.45 mg% subnormal. Van Eekelen, Emmerie and Wolff ('37) as

well as N e u w e i l e r ('39) claim that of the results obtained with the methods of these former authors, such as fall under 0.40 mg% are poor, scorbutic, and only those on a higher level than 0.80 mg% are sufficiently good, without saturation of the tissues. Values varying between 0.40 and 0.80 mg% are regarded by E e k e l e n *et al.* as fair, whereas N e u w e i l e r considers values between 0.40—0.55 mg% as hypovitaminotic and 0.60—0.75 mg% values as uncertain, hardly sufficient. P u r i n t o n and S c h u c k ('43), F i n c k e and L a n d q v i s t ('42) maintain that a vitamin C content of 0.80 or more in the plasma is the optimal vitamin C standard, whereas in the opinion of R a l l i *et al.* ('39) only 1 mg% and values surpassing it are optimal, 0.40 mg% being the lower limit of the normal area.

B i c k n e l l and P r e s c o t t (l.c. p. 511) emphasized that there is no clinical justification for the view that a level above 0.5 mg. to 0.7 mg. per 100 c.c. of blood is necessary for optimal health, and also that saturation is not a normal condition, but a definitely abnormal one since most individuals are not saturated on an average diet.

It has proved very difficult, on the basis of the present evidences, to decide what vitamin C concentrations in the blood are to be regarded as normal and what as pathological. Results of animal tests cannot be applied straight away to human individuals. Yet, it is hardly possible to disregard the fact that the vitamin C concentration in the blood plasma of animals capable of synthesizing it is as high as 0.6—1.0 mg% and even more, and the concentrations are equally high in the organs, in the rat e.g. even higher than in guinea-pigs on a natural diet, when the guinea-pig gets 12—30 mg. vitamin C per day, which is sufficient to maintain a level of about 1.0 mg% of vitamin C in the plasma (G i r o u d *et al.*, F o x and L e v y, E c k e r and P i l l e m e r, etc.) Therefore, the synthesis of ascorbic acid must be fairly high in most animals, but it does not seem logical to assume that they are forming excessive amounts of ascorbic acid and possess such a regulating system which maintains unnecessary high concentrations of ascorbic acid in their blood and organs, particularly as the tests show that larger doses of vitamin C administered to them are immediately excreted into the urine, thus evidently being in excess of the actual requirement. In the rat, which synthesizes ascorbic acid, tissues and organs are so unwilling to part with their ascorbic acid concentrations, that a 10 to 11 days' fasting on one hand, and massive doses of ascorbic acid on the other are powerless to bring about any change whatsoever in the ascorbic acid concentrations of the organs (S u r e *et al.* '39).

As far as man is concerned, there does not seem to be any reason to suppose that he is an exception among the highly developed animals and that the human organ- and cell-function had adapted themselves to essentially lower vitamin C concentrations. The evident fact that all primates have lost the faculty of producing in their body the vitamin C required by them indicates, that man's phylogenetic ancestors received food so rich in vitamin C, that their cells which had formed it — probably on receipt of more and more "telegrams" to reduce the production — had gradually adapted themselves to a minimum production and finally appearently discontinued it altogether. It

is probable that with an extension of the populated areas over the face of the globe, man has already in the course of thousands of years subsisted on food whose vitamin C contents were sometimes very scarce. It is hardly possible to demonstrate anymore whether the resistance to diminished supplies of vitamin C has increased in the course of time. It seems, however, that man has not regained his lost ability of a physiological vitamin C synthesis.

Ralli *et al.* ('39) demonstrated that the epithelial cells of renal tubules actively reabsorb the ascorbic acid filtered through the renal glomeruli up to a certain maximum amount, the surplus being excreted into urine. Organs have a pronounced tendency towards attaining a certain level of saturation with regard to ascorbic acid and they are capable of taking it from the blood, whose ascorbic acid concentration is much lower than that of the organs. This phenomenon must be regarded as an endeavour to revert to the disturbed normal condition and the state of saturation thus obtained, where the excretion into the urine is still comparatively scarce but abruptly increasing (about 3—5 mg%) is to be considered as an optimal state with regard to ascorbic acid. This optimal vitamin C standard is marked by the figure representing the ascorbic acid level in blood plasma, lying near the threshold value, slightly variable in different individuals and even in the same person at different times. Thus defined, the optimal vitamin C standard is of course not a clinical concept, but a physiological, biochemical one.

On the other hand, no correlation could be established between the patient's clinical condition and the vitamin C values in the plasma. These values alone have little or no diagnostic significance, even if they are low (Butler '42). In clinical manifestations of scurvy values of 0.0—0.30 mg% (Söderling and Hamne '37, Nielsen '38, Höygaard and Rasmussen '38, Ingalls '37, Wortis *et al.* '38), even of 0.40—0.50 mg% (Abt and Farmer '38) have been ascertained. On the other hand, several investigators found equally low spontaneous vitamin C levels in clinically healthy individuals (Herlitz '38, Dagulf '39, Nilsson '39, Westergaard '37, Rinehart *et al.* '38, Difs '40, Croft and Snorf '39, Butler and Cushman '40, etc.) In addition, several authors proved experimentally in human material that in a complete or practically complete lack of vitamin C, although its level in the plasma is reduced to zero, clinical symptoms characteristic of scurvy do not appear for a long time (Rietschel and Mensching '39, Crandon '40, Hunt '41, Farmer '44).

Butler and Cushman ('40), Crandon *et al.* ('40) etc. demonstrated that the whole blood still contains ascorbic acid, although its content in the plasma has gone down to zero, and they maintain that an estimation of ascorbic acid content in white cells and platelets yields a more reliable picture of the standard of ascorbic acid in the body, than determinations of the plasma ascorbic acid. The writer was, however, constrained to desist from estimations of ascorbic acid in the whole blood within the scope of this work.

Ahlborg and Brante ('40) found that the capillary resistance in healthy infants determined by Göthlin's method is abnormally weaken-

ed if the plasma vitamin C level falls below 0.30 mg%. Javert and Stander ('43) observed that newborn infants with a vitamin C value under 0.30 mg% in the plasma often developed hemorrhagic diathesis. Since hypoprothrombinemia is very common in the newborn but such low levels of vitamin C infrequent, they assume that vitamin C deficiency is partly responsible for hemorrhagic diseases in newborn infants. Bartlett, Jones and Ryan ('40, 42) demonstrated that the tensile strength in healing wounds was weakened if the plasma vitamin C level fell below 0.20 mg%. However, the evidence of these results is not conclusive, as Göthlin's method has been the object of much controversy, and Pijoan and Lozner ('44) were unable to support the observations made by Bartlett *et al.*

The minimum requirement of vitamin C of human individuals has been generally estimated at between 10 and 20 mg, (Schultzer ('37), Rietschel and Mensching (l.c.), Hamil *et al.* '38, Stefansson '39, Kellie and Zilva '39, Fox *et al.* '40, The Accessory Food Factors Committee of The Medical Research Council '48, etc.) The Technical Commission on Nutrition of the League of Nations has accepted 30 mg. as the minimum for protection against scurvy. In reality the minimum dose for young infants and adults may vary between 15 and 30 mg., since the investigations which are made on potatoes and other victuals, and on which the calculations of the daily intake of vitamin C are based, only determine the ascorbic acid. Yet potatoes and other vegetable products contain noticeable amounts of dehydroascorbic acid (Günther '42, Scheunert '40, etc.) which is reduced to ascorbic acid in the animal and human organism. According to Thysell ('39) and Medical Research Council ('48) a daily dose of 30—50 mg. vitamin C maintains under normal conditions a plasma vitamin C level of 0.20—0.40 mg%. According to investigations of The Accessory Food Factors Committee of The Medical Research Council ('48) "a plasma level of below 0.10 mg. per 100 ml., though an accompaniment of scurvy, is not proof of scurvy or of imminent scurvy." The investigations have been made on adult subjects. On the other hand, it is difficult to ascertain the earliest changes of subclinical human scurvy.

Under these circumstances the vitamin C level of 0.20 mg% in the blood plasma may be accepted as a limit value, and those falling below it should be regarded inadequate and an evidence of such a humoral-pathological condition of the body, which sooner or later will develop into subclinical or clinical scurvy. It should be noted that such an "incubation period" can be fairly long in an adult person and even last for years, depending on whether the substances and apparatuses (collagenous fibres, capillary and epithelial basal membranes, bone and cartilaginous tissue) whose formation was found to be affected in some unexplainable

way by vitamin C, are fully developed in the adult and only subject to continual renewal whose rate may depend on the strength of the wasting effect directed against the said connective and supporting apparatuses. It is evident that these supporting substances are relatively resistant in the adult individual, also with regard to the vitamin C deficiency.

As already mentioned, several investigators still consider the plasma vitamin C values 0.20—0.40 mg% poor and the values 0.40—0.60 subnormal, and up to 0.80 mg% suboptimal. In fact, the optimal vitamin C reserves of the body must be sufficient to secure an optimal vitamin C supply for the organs of the body, in all circumstances, and to guarantee the maintainance of the optimal capacities of the body, under all conditions, without occurrence of insufficiency.

It does not seem possible to determine, on the basis of the actual evidences, what real significance is to be attached to the plasma vitamin C values ranging from 0.20 to 0.60 mg%. They should therefore be considered at present as uncertain border values, until more exact knowledge as to their nature is gained. Taking the middle course, one could then characterize the values of 0.20—0.39 mg% as inadequate, with a question mark, and 0.40—0.59 mg% as relatively adequate, with a question mark.

In fact Szent-Györgyi ('34) has stated that between avitaminosis and perfect health there lies the extensive zone of hypovitaminosis.

CHAPTER IV

METHODS

The most reliable way to establish and to determine vitamin C is a biologic method, the guinea-pig test, which, however, consumes too much time and material to be suitable for clinical serial tests.

Attempts have therefore been made to find chemical means of rapidly and easily following up the changes of the vitamin C condition in the human organism under different circumstances. These chemical means are chiefly based upon the very considerable reducing power of ascorbic acid. There are numerous chemical bodies which ascorbic acid is able to reduce, however, it is a common characteristic of them all that they are not specific indicators of ascorbic acid but that a number of other reducing substances affects them in a similar way. Such substances, which under biological conditions have a disturbing influence on the determination of ascorbic acid, are certain sulphur compounds, such as thiosulphates, sulphides, cystein, cystin, glutathione, ergothionine and furthermore ferrosalts, tannic acid, uric acid, creatinin and the colour substances of urine. The main part of the reducing influence of these has been eliminated by titrating the ascorbic acid in a comparatively acid solution, with a pH of about 2.0—2.5 (Birch, Harris and Ray, '33). It was observed that the most suitable acid with the help of which the solution about to be examined can be oxidized is the metaphosphoric acid (Fujita and Iwatake '35, Bessey '38, Lehmann '47). The acid itself must not, of course, have an oxidizing effect neither upon the ascorbic acid or a reducing effect upon the reagent used.

By making numerous comparisons to the results obtained by the biologic method it was ascertained that mainly two colour substances must be regarded as the most suitable of all oxidizing substances known hitherto for the determination of ascorbic acid, i.e. 2,6-dichlorphenol-indophenol and methylene blue, the former being more generally used as an indicator of ascorbic acid. This titration method of vitamin C with the help of dichlorphenol-indophenol found by Tillmans ('27) originates from times, when the identity of vitamin C and ascorbic acid was still unknown.

Being a strongly reducing substance, ascorbic acid itself is an easily oxidizing substance whose stability, especially in pure water solutions, is slight. It is rapidly destroyed by oxygen and oxidizing ferments (ascorbic acid dehydrases, peroxidases) and mineral salts, which are present in different plant

and animal tissues. In acid surroundings, a milieu of hydrogen ions, such as solutions of acids, sour berries and fruit, it is preserved for a much longer time. Metaphosphoric acid was found to be a particularly effective stabilisator of ascorbic acid, as already mentioned. On the other hand, ascorbic acid is not stable in trichloracetic acid (Emmerie and van Eekelen '34, Fujita and Iwatake '35).

Yet a third aim is achieved by using metaphosphoric acid as a stabilisator, i.e. the elimination of proteins which is indispensable when determining ascorbic acid in plasma, organs, and in other material containing protein. Metaphosphoric acid is considered to be a very suitable deproteinisation substance (Nielsen and Hjärde '41, etc.).

When using dichlorphenol-indophenol (TR) as a reagent of ascorbic acid, it must be remembered that it is a fairly labile compound and that the titre of its aqueous solution changes rather quickly, especially at usual room-temperature. On the other hand, the titre of a reagent which has been kept in a refrigator can remain unchanged for a longer time. The most reliable way would be to control the titre of the TR-solution once daily. This can be done for instance by titrating a 1 mg% ascorbic acid solution with a reagent solution. This method requires not only chemical scales but also a constant supply of 100% pure ascorbic acid. On the other hand, the iodometric control of the purity of ascorbic acid renders its determination still more difficult. Some workers have considered sufficient to compare the titre of the reagent solution once a day with a solution prepared from the ordinary vitamin C tablets, obtainable on the market. Of course such a method of comparison is not fully reliable. In order to facilitate the determination of ascorbic acid as much as possible the Swiss firm Hoffmann-La Roche has put on the market dichlorphenol-indophenol tablets and each tablet corresponds to 1 ± 0.05 mg. ascorbic acid. However, according to Dagulf ('39) the error may rise to 10%.

A source of error to be taken into consideration when determining the vitamin C by usual chemical ways is the circumstance that only the ascorbic acid is demonstrated in this way, while its first stage of oxidation, the dehydroascorbic acid which has been shown to possess considerable antiscorbutic effect remains altogether undetermined, as it does not reduce TR-solution. Emmerie and van Eekelen ('34) use hydrogen sulphide in order to re-reduce dehydroascorbic acid. Before titration of the ascorbic acid the hydrogen sulphide must, however, be carefully eliminated by carbon dioxide, as otherwise it will also reduce the TR-solution. The use of hydrogen sulphide can easily become a new source of error and at any rate it makes the method so complicated that it is not applicable in the performance of serial tests. Furthermore, animal tissues possess the capacity to reduce dehydroascorbic acid (Holz and Koech '42), which is therefore found less frequently and in smaller quantities in animal tissues than in plants.

Günther ('42) demonstrated an electrolytic method in order to reduce the dehydroascorbic acid. Nagayama *et al.* ('40, '41) eliminated in their

determinations of ascorbic acid in the urine the interfering colour substances with the help of phosphotungstic acid. G u n s a l u s and H a u d ('41) used a suspension of bact. coli for the reducing of dehydroascorbic acid.

The Methods Employed in the Present Investigation

The blood specimen was taken in the following way: a small amount of 20% potassium oxalate solution was drawn into a syringe of 5 ml. moistening its walls. The piston was pushed to the bottom so that a little potassium oxalate remained in the nozzle of the syringe, a small quantity, but sufficient to prevent the coagulation of the blood. 5 ml. blood were drawn into the syringe from a cubital vein and this blood was immediately placed into a small centrifuge tube. 20 mm^3 of blood was pipetted off at once for the determination of hemoglobin and the tube was placed into a centrifuge. The blood specimen was taken in the morning, before a meal.

Determinations of ascorbic acid in the plasma were performed in the following way, according to the modified method of F a r m e r and A b t ('35) which is based upon the methods of T i l l m a n s (l.c.) and F u j i t a and I w a t a k e (l.c.). Into a small, completely dry centrifuge tube

2 ml. 5% metaphosphoric acid (MPA)
1 ml. plasma and with the same pipette
1 ml. aq. dest.

were measured and mixed and centrifuged. 2 ml. of centrifugate were pipetted into a completely dry small bowl and titrated by dichlorphenolindophenol solution, the titre of which was 1 ml. = 0.02 mg. ascorbic acid.

The actual titration was performed by using a comparative colour solution in the following way: into a small white bowl 2 ml. of 2.5 % metaphosphoric acid were measured, and from a microburette 0.08 ml TR-solution were introduced, which solution gives a scarcely perceptible, slightly red tint to the MPA-solution. The solution under examination was then quickly titrated by dropping from the burette at first 0.08 ml. of reagent and then adding more, if necessary, until a slight colour remained, persisting for 25 seconds, with a strength equal to that of the comparative solution.

The titration must be performed quickly within 25 seconds, because the red TR-solution changes to a lighter shade fairly rapidly if the pH of the solution is below 2.2 as is the case in this method (B i e r r i n g '47.) Nor does the slower reducing power of the sulphhydryl compounds interfere, if the titration is rapid enough (M i n d l i n and B u t l e r '38, B ø e '47). This non-resistance of the TR-colour below pH 2.2 has consequently an effect increasing the values. Since, however, in the method used by me the change of the TR-colour takes place also in the comparative solution into which the TR-reagent has been added only a few seconds previously to the performance of the actual titration, and when the blank value is subtracted, no errors due to inconstancy of the red TR-colour appear through the use of this method. This is also indicated by the presence of zero values in the plasma.

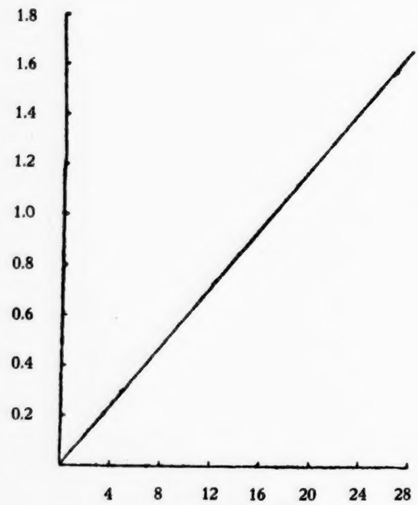


DIAGRAM 6.
Titre Line for Dichlorphenol-indophenol.

Abscissa: Ascorbic acid (in γ) equivalent to 1 ml. of dichlorphenolindophenol solution.

Ordinate: Extinction.

e = 0.765 = 0.0129 mg. ascorbic acid per 1 ml.
e = 1.185 = 0.020 „ „ „ „ „ „
e = 1.715 = 0.029 „ „ „ „ „ „
Cuvette 0.5 cm.
Filter S 61.

Calculation: for instance, 0.11 ml. of TR-solution have been used. The non-reduced TR-quantity employed for a determination of the titration limit, or the blank value 0.08 ml. is deducted, there remain 0.03. This value multiplied by four directly gives the ascorbic acid content in mg%. Thus in the case taken as example the result will be 0.12 mg%.

The deduction of the blank value is necessary because otherwise too high values would be obtained and zero values would not appear at all which by some authors (R i e t s c h e l and M e n s c h i n g '39) are considered to belong to the laboratory findings of scurvy, recently manifested and still untreated. On the other hand, these zero values may also appear in cases of scurvy which has not yet broken out, and even in clinically healthy persons (D i f s '40).

In my determinations of ascorbic acid performed in the years 1939—41 under peace conditions, I have controlled the titre of the dichlorphenol-indophenol solution by using a solution prepared from crystalline l-ascorbic

acid. I then have prepared the dichlorphenol-indophenol solution and controlled its titre in the following way: first, a stock solution is prepared which is somewhat stronger than the one which will be used for the titration, by dissolving about 50 mg. dichlorphenol-indophenol powder (Hoffman-La Roche) in 500 ml. buffer solution which contains 1,59 g. primary and 3,86 g. secondary phosphate (S ö r e n s e n). The pH of the buffer solution is 7.07. By diluting this stock solution with distilled water a titration solution was prepared daily which, determined with the help of the photometer of Pulfrich with a cuvette of 0,5 cm and an S 61 filter, gives the extinction 1.185. In this case 1 ml. of TR-solution corresponds to 0.02 mg. ascorbic acid. The titre line in diagram 6 shows relation between extinction and titre.

Determinations of ascorbic acid in the urine were performed according to the slightly modified method of N a g a y a m a *et al.* ('40, '41) in the following way: into the centrifuge tube are measured:

2 ml. 5% metaphosphoric acid
1 ml. urine and
1 ml. 5% phosphotungstic acid,

these are mixed and centrifugated. 2 ml. of clear centrifugate are pipetted into a small bowl and titrated with dichlorphenol-indophenol until a slightly reddish shade is obtained. Depending on the quantity of TR-solution employed 0.08—0.20 ml. are deducted as blind value. The calculation is the same as in the determination of plasma ascorbic acid.

Determinations of vitamin C in food were performed according to the same principles as in plasma. About 1—5 g. victuals, the berries whole, were weighed and then crushed in a mortar with sand and metaphosphoric acid, carefully and without boiling. The final dilution was performed in the mortar with 5% metaphosphoric acid and distilled water at a proportion of 1: 2, 1: 4, 1: 10, 1: 20, 1: 50, 1: 100 or 1: 200 depending on vitamin C content of the substance to be investigated. The final concentration of metaphosphoric acid was 2.5 %. Carrots and apples were diluted 1: 10, potatoes, rape, rutabaga, peas, gooseberries, currants 1: 10 or 1: 20, raspberries, strawberries, cloudberries, orange juice 1: 50 or 1: 100, even 1: 200, soups and broth 1: 2, milk 1: 4 and so on. After the centrifugation 2 ml. of clear solution were pipetted into a small bowl and titrated with dichlorphenol-indophenol solution, whose titre was 1 ml. = 0.02 mg. ascorbic acid.

In order to perform the titration rapidly enough it is very appropriate in all determinations of vitamin C to titrate a small quantity (1—2 ml.) of the solution sufficiently diluted. Should the dilution, owing to an unexpected high vitamin C content, not be sufficient and should the consumption of the reagent solution be great, the dilution must be performed once more so that the consumption of the TR-solution does not considerably exceed 2 ml. A sufficient dilution is necessary also because the TR-solution I used diminishes the acidity of the solution to be titrated, as it contains a buffer substance

and might even completely neutralize it, should it become necessary to use more than 5 ml. in proportion to 2 ml. of the solution to be examined and having a 2.5 % content of metaphosphoric acid.

With regard to the reliability of the method of F a r m e r and A b t it was found to be satisfactory in the control determinations which have often been performed e.g. in the Department of Medical Chemistry of the University of Helsinki. Titration with TR-solution gives clearly higher values than the methylene blue method of L u n d and L i e c k (B r a e s t r u p '39, D a g u l f '39, K i r k and W a r b u r g '40, T r i e r '40, O t t s e n '42). It must be admitted that the method of F a r m e r and A b t contains possibilities of error, but I am of the opinion that if these are constantly kept in mind, this method yields comparatively satisfactory results.

In Finland H o r e l l i ('37) employed the method of T a u b e r and K l e i n e r ('35) for the performance of urine analyses in tuberculous patients. Although there is no complete certainty about specifity of the ascorbinases (T r i e r '40), the enzymatic method is nevertheless considered to be the most specific of the chemical determination methods of ascorbic acid used at present (L e h m a n n '47). I was, however, unable to make use of it as it would have involved more difficulties under war-time conditions and as the plasma ascorbic acid determinations do not seem to be noticeably interfered with by non-specific reducing substances. The numerous zero values of the plasma testify that there is either a negligible quantity of non-specific reducing substances or that they do not affect the TR-reagent, or that their quantity, should there appear more of them in a higher vitamin C content of the plasma, diminishes parallel with the diminishing of the quantity of ascorbic acid to quite an insignificant part. One must, however, apparently, presume that the non-specific reducing substances of urine originate from the blood even in these cases where they cannot be demonstrated in the plasma by TR-reagent.

When comparing titre values obtained by chemical methods with the results of the biologic method, a satisfactory correspondence between them was found. This was also ascertained by S c h e u n e r t and collaborators ('40) with regard to the vitamin C content of the potato.

CHAPTER V

AUTHOR'S INVESTIGATIONS

As stated earlier in Chapter III the concentration of ascorbic acid in blood plasma of a human being is dependent in the last place mainly on the ascorbic acid amount daily obtained in the diet. As the ascorbic acid content of foods decreases during the winter and spring, especially in northern countries, this change is reflected in the ascorbic acid level of the blood plasma. In several countries the vitamin C status of the population has been investigated and even great variations have been found in the vitamin C concentration of the blood plasma in different seasons of the year (Trier '40, Raunio '41, Ebbesen and Rasmussen '44, Hagtvet '45, Andersen '45, Wingren '46, Tötterman '49, etc.).

In treating my material I have estimated the ascorbic acid standard of the investigated according to the following rules, the exposition of which has been given in Chapter III:

The plasma ascorbic acid values	0—0.19 mg%	— subnormal (inadequate) ascorbic acid standard
—,,—	0.20—0.39 mg%	— uncertain border values indicating inadequate standard
—,,—	0.40—0.59 mg%	— uncertain border values the adequacy of which is questionable
—,,—	0.60—0.74 mg%	— relatively adequate ascorbic acid standard
—,,—	above 0.75 mg%	— optimal ascorbic acid standard

My material covers in all:

92	Prisoners from the years 1939—41
283	Soldiers from the years 1940—43
123	Civil persons from the years 1940—43
498	Persons in total.

1. *Ascorbic Acid Content of the Blood Plasma of Prisoners*

The results of the ascorbic acid determinations on prisoners have been presented in tables 12 and 13. The ascorbic acid concentration of the plasma was in the year 1939 in the prisoners of Helsinki Central Prison and in 1940 in the prisoners of Karvia Reserve Prison still in the beginning of August very low, in average 0.12 mg %. In the late winter and the spring of 1941 the ascorbic acid concentration of the plasma varied from 0.0 to 0.64 mg% and was in average 0.20 mg%. In estimating the physiological adequacy of these ascorbic acid concentrations according to the norms given before, it can be seen from table 13 that in 50 % of the investigated prisoners the ascorbic acid standard was on a subnormal level from February to August and that in addition in 41.3 % the ascorbic acid standard was unsatisfactory rather than distinctly adequate. Only in 8.7 % of the investigated cases the ascorbic acid concentration of the plasma was adequate. Although there are no determinations with regard to the period of time from May to June, it can be assumed that the average ascorbic acid standard hardly could have been essentially different. It must be added, however, that this vitamin C standard is on the same level as that of the free population in the late winter and higher than in the group of investigated soldiers during the first half of the year 1942.

Instead, although there are no investigations regarding the time between August and February, it can be assumed on the basis of the investigations made on other groups of the population, that following to the new potato crop there appeared increase in the quantity of the ascorbic acid of the prison dietary increasing the ascorbic acid standard of the prisoners in a corresponding degree. The difference with regard to the population living in freedom is nearest in the fact that in the majority of private households transfer over to the use of the new potato crop takes place already in July, latest in August, while in prisons because of mass-provisioning the use of early potatoes is lacking the transfer over to the new potato crop taking place only at the end of September or in the beginning of October. Due to the nature of mass-provisioning new potatoes do not come on the table of the prisoners immediately

TABLE 12.

The Ascorbic Acid Concentration of the Blood Plasma in Male Prisoners in the Years 1939—1941.

Place of Investigation	Time of Investigation	Number	Ascorbic Acid Concentration of the Plasma	
			Range mg%	Average mg%
Helsinki Central Prison	1939 26/7—17/8	10	0.0 —0.24	0.12
Karvia Reserve Prison	1940 28/7—6/8	5	0.04—0.20	0.12
—„—	1941 26/2—26/4	77	0.0 —0.64	0.22
Total or Average		92	0.0 —0.64	0.20

TABLE 13.

The Vitamin C Status of the Prisoners in the Years 1939—1941 Estimated on the Basis of Ascorbic Acid Concentrations of the Blood Plasma.

Place of Investigation	Time of Investigation	Number	Ascorbic Acid Standard							
			Subnormal 0.0—0.19 mg%		Border cases				Normal 0.60 mg% and above (Optimal 0.75 mg% and above)	
					Subnormal? 0.20—0.39 mg%		Normal? 0.40—0.59 mg%			
			Number	%	Number	%	Number	%	Number	%
Helsinki Central Prison	1939 26/7—17/8	10	8	80	2	20	—	—	—	—
Karvia Reserve Prison	1940 28/7—6/8	5	4	80	1	20	—	—	—	—
—„—	1941 26/2—26/4	77	34	44.2	35	45.4	7	9.1	1	1.3
Total or Average		92	46	50.0	38	41.3	7	7.6	1	1.1

after they have been dug up from the ground as is the case with early potatoes in private households. In the prisons new potatoes have always been stored for a week or two. The vitamin C effect of such potatoes is not any more as high as that of the potatoes which can be cooked immediately after digging and in which the ascorbic acid content may be above 30 mg%. Accordingly, besides the fact that the new potato crop which is richer in vitamin C comes at the disposal of the prisoners more than two months later than in free households, the vitamin C effect of same is not even in the beginning as intensive and does not last strong as long as the effect of the summer potato directly consumed after digging in private households. Thus the season of insufficient vitamin C service is about two months longer with regard to prisoners than with regard to free people. The season of sufficient vitamin C service which for the free population usually covers the time from the end of July to the end of December, lasting thus for 5 months, begins with regard to prisoners toward the end of September lasting only for about 3 months.

Kyhos *et al.* ('44) have stated that during the winter and spring months already since October, the ascorbic acid concentration of the blood plasma of the prisoners decreased in spite of the fact that the intake of the ascorbic acid was kept unchanged (50 mg. daily). The said investigators stated also that on the usual prison dietary the ascorbic acid level remained below 0.20 mg% in other months except in August and September when it increased up to 0.25 mg%. Owing to the fact that in the Finnish prisons supply of vitamin C is improved due to the new potato crop at the point of time when the climatic factors begin to cause increase in the consumption of the ascorbic acid, in all probability the ascorbic acid concentration, under conditions in the Finnish prisons, will never rise to the same level as among the free population.

Vitamin C Content of the Prison Dietary

The quantities of various food-stuffs included in the prison dietary valid in the year 1941 contained in the spring of 1941 about 70 mg. ascorbic acid per day and man. However, due to the way of preparing the prison food the prisoners obtained in reality only

about 50 % of the ascorbic acid quantity which originally was existing in the food articles used. Thus the prison milk contained in March—April 1941 in average 0.88 mg% of ascorbic acid, so that the prisoners obtained daily 4—5 mg. ascorbic acid in milk. The scant ascorbic acid content of the milk was due to the fact that 2/3 of the milk was separated and fairly long stored, the dairy being distant from the prison. The potatoes used in Karvia Reserve Prison contained in March—April 1941 in average 8 mg% of reduced ascorbic acid. The prisoners obtained in the cooked unpeeled potatoes included in their supper about 20—25 mg. of ascorbic acid, whereas the meat and fish soups contained only about 0.7—1.0 mg% of ascorbic acid. Thus the prisoners obtained in their soups only about 10 mg. of ascorbic acid daily, or in total 35—40 mg. of ascorbic acid per day.

The prison dietary confirmed in the year 1945 contained considerably more of rutabaga. Owing to this fact the prisoner's daily food contained in the autumn 150 mg. and in the winter about 100 mg. of vitamin C. It can be considered that the prisoner's nutriment even then contained about 50 % of the vitamin C amounts mentioned above.

2. *Ascorbic Acid Content of the Blood Plasma of Soldiers*

In tables 14 and 15 have been presented the results of the ascorbic acid determinations of blood plasma on soldiers. Some already in the year 1940, in July—August made determinations on soldiers of the air flotilla placed at Karvia showed that in persons using a standard dietary, even in peace time, easily as late as in August, the vitamin C standard can remain low. According to the investigations made by me during the war, in the years 1942—43 in Käkisalmi garrison, the ascorbic acid standard of the soldiers remained during the whole first half of the year on a subnormal level (0.08 mg%), showing no marks of increase before August in the year 1942. Thereafter the ascorbic acid concentration of the plasma remained above the limit of subnormal until the end of the year, the monthly values varying between 0.20 and 0.49 mg%. The monthly minimum value of the ascorbic acid concentration appeared in March (0.04 mg%) and the maximum value in October (0.49 mg%). Zero values were discovered in the year 1942 in 32 cases (16.8 %), viz. in the course of February—June. In the year 1943 there was only one zero value, viz. in April.

From table 15 can be seen that in all my soldier material the spontaneous ascorbic acid standard was on a subnormal level in 65.7 % and that in not more than 3.9 % of the cases can be considered to have been on a physiologically adequate level, the uncertain adequate cases included, at the most in 11.0 %. In first half of the year 1942 the ascorbic acid standard was at its lowest. At the same time the potato supply situation was worst. Due to a comparatively rich yield of berries and to nev potatoes the vitamin C standard increased samewhat in the course of August (0.25 mg%), more clearly, however, in October (0.49 mg%). Even at that time the average ascorbic acid standard did not rise on a physiologically satisfactory level.

TABLE 14.

The Ascorbic Acid Concentration of the Blood Plasma of Soldiers in the years 1940—43.

Year Month	1940		1941		1942				1943			
	Number	mg %	Number	mg %	Number	Average Monthly Value mg %	Average Quarterly Value mg %	Average Half Year Value mg %	Average Monthly Value mg %	Average Quarterly Value mg %	Average Half Year Value mg %	Number
I					11	0.08			0.13			8
II					46	0.08	0.07		0.14	0.16		5
III					19	0.04			0.20			9
IV					7	0.10		0.08	0.16		0.17	12
V					9	0.10	0.09		0.18	0.17		18
VI			6	0.9	15	0.09			0.17			12
VIII		0.19			4	0.10			0.56			4
VII	6	0.07			20	0.25	0.22		0.43	0.49		4
IX	3				9	0.20		0.29				
X					9	0.49						
XI					26	0.32	0.33					
XII					21	0.27						
	9		6		196	0.17			0.20			72

TABLE 15.

The Vitamin C Status of the Soldiers in the Years 1940—1943 Estimated on the Basis of the Ascorbic Acid Concentrations in the Blood Plasma.

Place of Investigation	Time of Investigation Month and Year	Number	Ascorbic Acid Standard							
			Subnormal 0.0—0.19 mg %		Border cases				Normal 0.60 mg% and above (Optimal 0.75 mg% and above)	
					Subnormal? 0.20—0.39 mg%		Normal? 0.40—0.59 mg%			
			Number	%	Number	%	Number	%	Number	%
Karvia	VII-VIII/'40	9	7		2					
Luumäki	VI/'41	6	6							
Käkisalmi	I-VI/'42	107	100	93.5	5	4.7	2	1.8		
„	VII-XII/'42	89	33	37.1	31	34.8	17	19.1	8(3)	9.0(3,4
„	(I-XII/'42	196	133	67.8	36	18.4	19	9.7	8(3)	4.2)
„	I-VIII/'43	72	40	55.5	28	38.9	1	1.4	3(3)	4.1 (1,5
Total or Average		283	186	65,7	66	23,3	20	7,1	11(6)	3,9(2,1)

Vitamin A and C Content of the Soldiers' Food

The daily ration of provisions of the Finnish soldiers contained in the years 1940—43 the following quantities (g.) of different food-stuffs:

	1940—41	1942—43
Dry bread (fresh)	400 (550)	350 (480)
Grain or meal	80—100	100
Sauce flour	20	20
Fresh potatoes	800	800
Butter	40	20
Cheese	40	30
Margarine or lard for food preparation	20	10
Fresh beef	125	100
Milk	400	200
Tea	1—1/2	1/2
Sugar	40	40
Salt	10	10
Spices	5	5

In lack of primary constituents or in order to gain variety the above mentioned food-stuffs could be exchanged for other food-stuffs, e.g. fresh potatoes for peas, macaroni, grain, dried potatoes, dried or fresh root-crops or cabbage.

Simola ('41) is of the opinion that a daily potato ration of 800 g. is sufficient to cover the daily vitamin C requirement very well in the autumn and nearly satisfactory in the spring and summer seasons, provided that the potato is handled in a right way in the preparation of food. When the loss of peels and of vitamin C in the food preparation are taken into consideration, it can be reckoned that the soldier's ration of provisions contains in the autumn somewhat more than 90 mg. and in the spring and summer seasons nearly 50 mg. of vitamin C, as far as a good quality of potato is at disposal (Simola).

During the war the exchange table had to be used very often, among others potatoes had to be exchanged for peas. In addition it was very difficult to protect the potatoes from cold. The vitamin C content of potatoes damaged by cold decreased very steeply due to the fact that the potatoes thawed in between.[1]) In the preparation of food a greater vitamin C loss was brought about therethrough that damaged potatoes could not be cooked unpeeled but must be stewed, mashed or baked in dish. The vitamin C content of frozen potatoes could occasionally be very low immediately after cooking them unpeeled. In the twelve determinations performed during the period of time from January 29th to February 5th, 1942 the ascorbic acid content of the potatoes cooked unpeeled varied between 0.1 and 2.45 mg%, being in average 1.36 mg%. The ascorbic acid content of mashed potatoes was zero. Owing to this the vitamin C intake of the soldiers during the war was now and then very scant. As a result of the scant intake of ascorbic acid the vitamin C standard of the soldiers fell during the winter lower than usually, and also clinical symptoms indicating vitamin C deficiency, such as hemorrhagic gingivitis, appeared among the soldiers. These will, however, be described in another paper.

Together with the decrease of the soldiers' milk and butter rations in 1942, the vitamin A content of the soldiers' provisions also de-

[1]) The freezing and preserving as frozen do not decrease the vitamin C content of the food-stuffs (Scheunert '40, Simola '41).

creased noticeably. The daily ration contained in the winter about 645 I.U., in the summer 1335 I.U. of vitamin A. The amount of carotene varied according to the availability of vegetables containing carotene. The vitamin A intake of the soldiers was nevertheless greater due to the extra milk received from the civilian population and to the food parcels from home, as well as to the occasional vitaminization of butter.

3. *Ascorbic Acid Content of the Blood Plasma of Civil Persons*

Earlier investigations concerning the vitamin C status of the Finnish people generally have resulted in the opinion that same is fairly good, mainly because of the rich use of potato (Leppo '39, The People's Nutrition Committee '40). According to the investigations made by the PNC (l.c. pages 83—86) 65 % of the families investigated in the years 1936—37 had obtained in their food enough of vitamin C, 35 % had received less than the requirement value used implied.[1]) The PNC (pages 87—88) considers that the lack of vitamin C is due "before all to the scantiness of food, accordingly to quantitative subnutrition and not so much to the wrong composition of the food."

In tables 16 and 17 have been demonstrated the results of the determinations of the ascorbic acid of the blood plasma among the civilian population at Karvia community and among the war emigrants who had returned to the town of Käkisalmi and its surroundings. From table 16 it becomes evident in some degree that also in freely chosen dietary the average vitamin C standard of the population at Karvia community remained on a subnormal level in the first quarter of the year 1941, rising toward the end of April up to 0.24 mg%, but decreasing again somewhat in May. In case the improvement of the vitamin C standard in the latter part of April was not an accidental phenomenon depending on the restricted number of cases, it might be supposed that it was dependent on the possible decreasing consumption of vitamin C due to warmer weather. It could not be a result of Easter oranges, as none were distributed at Karvia. The vitamin C standard of the population was on its highest from August to November, decreased distinctly in December and fell in January below the borderline of the subnormal.

It can be seen from table 17 that in a total of 42.3 % of the investigated cases the vitamin C standard was subnormal and only in 20.3 % the standard was physiologically satisfactory. The rest were uncertain border cases, of which in 11.4 % the vitamin C reserve was such as to maintain full health in favourable circum-

[1]) 50 mg. = 1,000 I.U. for adults and children over 10 years of age, 25 mg. = 500 I.U. for children under 10 years of age.

TABLE 16.

The Ascorbic Acid Concentration of the Blood Plasma of the Civilian Population in the Years 1940—1943.

Time of Invest-igation Year and Month	Karvia				Time of Invest-igation Year and Month	Käkisa'mi			
	Number	Average Monthly Value mg %	Average Half Year Value mg%	Average Annual Value mg%		Number	Average Monthly Value mg%	Average Half Year Value mg%	Average Annual Value mg%
1940					1942				
VII	3	0.26			II	3	0.11		
VIII	14	0.55			III	3	0.17	0.10 mg%	
IX	1	0.48	0.48 mg%		V	2	0.04		
XI	1	0.24			VI	6	0.09		
1941					VIII	14	0.55		0.40 mg%
I	3	0.13		0.29 mg%	IX	5	0.54		
II	8	0.16			X	7	0.82	0.51 mg%	
III	4	0.11	0.19 mg%		XI	17	0.41		
IV	10	0.24			XII	5	0.26		
V	12	0.22			1943				
					I	2	0.22		
					V	3	0.17		
	56					67			

stances of life. But in 26.0 % the vitamin C reserve was so low that it in all situations of life hardly is sufficient to secure an optimal vitamin C function in all the organs and tissues of the body.

In the first half of the year the ascorbic acid standard was on a normal level in no of the investigated cases. There were 7.1 % of uncertain normal cases. The percentage of subnormals was 66.1 % and that of uncertain subnormal cases 26.8. In the latter half of the year a subnormal ascorbic acid standard was found in 22.4 % of the investigated, and in addition 25.4 % with an uncertain subnormal standard. There were 37.3 % of normal and 14.9 % of uncertain normal cases. Thus nearly one half of the persons investigated (47.8 %) had not receaved enough of vitamin C. The different vitamin C standard of the people in the latter half of the year when the vitamin C content of the vegetables is comparatively high even in Finland, might be partly due to the different alimentary habits and partly to the different vitamin C requirements of the people.

In saturation tests, which I shall explain in another connection, it has become clear that the ascorbic acid absorbs readily, but that

TABLE 17.

The Vitamin C Status of the Population of Karvia Community in the years 1940—41 and that of the War Emigrants Who had Returned to the Town of Käkisalmi and its Environs in the Years 1942—43.

Place of Investigation	Time of Investigation	Number	Ascorbic Acid Standard							
			Subnormal 0.0—0.19 mg%		Border cases				Normal 0,60 mg% and above (Optimal 0.75 mg% and above)	
					Subnormal? 0.20—0.39 mg%		Normal? 0.40—0.59 mg%			
			Number	%	Number	%	Number	%	Number	%
Karvia	VII-XI/'40	19	5	26.3	5	26.3	3	15.8	6 (6)	31.6
"	I-V/'41	37	22	59.5	11	29.7	4	10.8	—	—
Käkisalmi	II-VI/'42	14	12	85.7	2	14.3	—	—	—	—
"	VIII-XII/'42	48	10	20.6	12	25.0	7	14.6	19 (14)	39.6 (29.2)
"	I, V/'43	5	3	60.0	2	40.0	—	—	—	
Total or Average		123	52	42.3	32	26.0	14	11.4	25 (20)	20.3 (16.3)

the quantities required to maintain a certain ascorbic acid concentration of the blood plasma vary considerably in different persons.

On the basis of present knowledge it is not possible to decide whether and in what degree the physiologically inadequate ascorbic acid standard has any influences upon the various diseased manifestations which generally occur among civilized peoples. It is noteworthy, however, that the ascorbic acid in most animals probably belongs to the group of so-called tissue hormones and that only the human being and a few animals have become dependent on an intake of vitamin C. The similarity of the life-processes of the human beings and the mammals who synthesize vitamin C, compels one to suppose that approximately the same ascorbic acid concentrations are needed in order to maintain the optimal cell-function as well in organs and tissues of the human being as those of animals. Therefore, the great variations which are noted in the vitamin C standards of humans in different seasons of the year in Finland as well as in other countries, among civilized peoples, cannot be considered a normal and favourable phenomenon regarding the development of the public health.

The physiologically inadequate vitamin C supply concerns not only adults but also children. The vitamin C should be considered as the growth-vitamin of the mesenchyma, the importance of which for the growing organism is evident. Therefore it should be taken up for more detailed deliberation, how these common but unphysiological variations in the vitamin C standards in different seasons of the year could be levelled in an economically most profitable and physiologically most satisfactory manner.

The dietary of the people is essentially dependent on the foodstuffs produced in the country. This production should be more than before influenced by the sanitary views. As the potato in the winter and early summer is not enough to maintain adequate vitamin C standard, the cultivation of root vegetables which are richer in vitamin C, such as turnips and swedes, should be increased and possibly new kinds developed. Because of the same reason horticulture ought to be increased and preservation methods developed.

About the significance of the protective nutrients for the health of people more effective propaganda work should be done and information ought to be distributed before all among the housewives in order to have the one-sided and in many respects inadequate diet of the Finnish people amended.

4. *Comparison between the Ascorbic Acid Content of the Blood Plasma and that of the Urine*

Several investigators (Wieters '35, '37, Lund '37, etc.) are of the opinion that no vitamin C whatsoever is normally excreted in the urine. Roe and Hall ('39) stated by employing a new method that also the urine of 28 % of healthy persons lacked ascorbic acid. Wieters has proved that the urine of a human being does not affect the scurvy of the guinea-pig. That instead in saturation state vitamin C is excreted through the kidney has been stated both biologically (Johnson and Zilva '34) and by isolating crystalline ascorbic acid from the urine (Wieters '35). The ascorbic acid which has been found in urine only in reduced form (Johnson and Zilva) thus belongs to the subtances which have a certain renal threshold. According to Baumann ('37) the renal threshold of ascorbic acid is 1.0—1.4 mg%, according to Lund 0.75—1.2 mg%, according to Faulkner

TABLE 18.

Averages of the Reduction Values, Specific Gravity and pH Values of the Urine in Different Vitamin C Concentrations of the Blood Plasma.

Vitamin C Concentration of the Blood Plasma mg%	Number	Urine		
		Reduction Value in Average	Specific Gravity in Average	pH in Average
0.0	36	0.90 ± 0.11	1025	5.9
0.0 —0.09	124	0.97 ± 0.05	1026	5.9
0.10—0.19	99	1.05 ± 0.05	1022	5.8
0.20—0.29	72	1.23 ± 0.05	1023	5.9
0.30—0.39	28	1.39 ± 0.09	1024	5.8
0.40—0.49	34	1.41 ± 0.09	1024	6.0
0.50—0.59	12	1.51 ± 0.12	1024	5.8
0.60—0.69	17	1.56 ± 0.09	1022	6.0
0.70—0.74	7	1.68 ± 0.22	1023	5.7
0.75—0.79	8	3.79 ± 1.1	1026	5.7
0.80—0.89	26	4.45 ± 0.7	1023	5.7
0.90—0.99	17	4.42 ± 0.6	1021	5.7
1.00—1.09	24	6.54 ± 0.9	1023	5.7
1.10—1.19	11	7.03 ± 1.0	1021	5.9
1.20—1.29	19	6.93 ± 0.6	1025	5.8
1.30—1.39	13	11.56 ± 2.2	1022	5.9
1.40—1.72	10	16.15 ± 3.6	1025	5.8
	521		1023	5.8

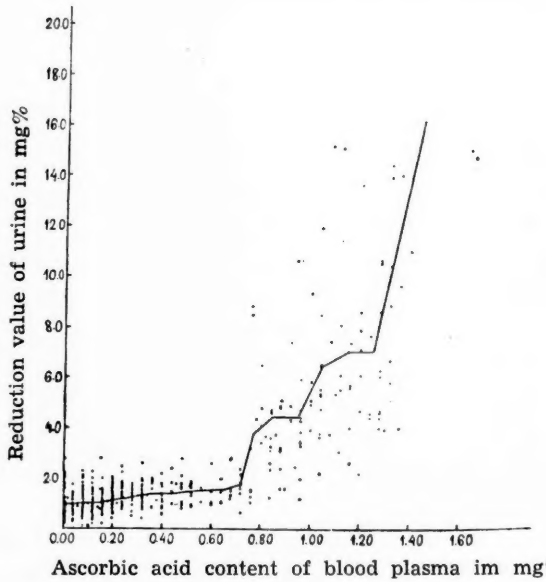


DIAGRAM 7.
Relation between Reduction Values of Blood Plasma and Urine.

and T a y l o r ('38) 1.4 mg% in adults, according to B r a e s t r u p 0.8—1.6 mg% in infants, to mention only a few results. The threshold value nevertheless is not quite constant even in the same person (K e l l i e and Z i l v a '39), varying considerably in different individuals (T r i e r '40).

In order to find out in which degree conclusions could be drawn on the ground of vitamin C determinations of the urine with regard to the vitamin C concentration of the blood and the vitamin C standard of the organism, I have in connection with the vitamin C determinations of the blood plasma also determined the reduction value of the urine. The results of these determinations, are presented in table 18 and depicted graphically in diagram 7.

As appears from table 18, on the plasma vitamin C concentration range of 0.0—0.74 mg%, only minimal tendency to rise can be observed in the reduction values of the urine. Instead, above 0.75 mg% a distinct rise can be observed. It is likely that the reduction values of the urine which have been obtained when the blood plasma has not contained ascorbic acid in an ascertainable degree, are not due to ascorbic acid but to other reducing substances. Accord-

ing to my determinations 0.16—2.76 mg% of these substances appeared in the urine, in average 0.90 ± 0.11 mg%, and M+3 σ = 2.85 mg%. Reduction values above 2.0 mg% appeared mostly in such cases where the specific gravity was above 1030 as is often the case in fever. A high specific gravity nevertheless does not always cause a high reduction value. Whether the evenly increasing reduction values of the urine which correspond to the increasing plasma vitamin C conocentrations 0.0—0.74 mg% must be considered as a sign of an excretion of ascorbic acid or whether the increase of the values is dependent on the increased excretion of other reducing substances in connection with the improvement of the vitamin C standard, cannot be established without biological tests. The excretion of the ascorbic acid, in case such takes place, then is relatively small, generally considerably below 0.80 mg%.

On the other hand, it is apparent that the sharp rise in the reduction values of the urine above the plasma vitamin C concentration value of 0.75 mg% (diagram 7) is due to ascorbic acid. Even in this case an inspecific component must be considered to be included in the reduction value of the urine which probably is of the same greatness as the reduction values of the urine when still no excretion of the ascorbic acid into the urine takes place, or about 1.0 mg%. When the daily excretion of the ascorbic acid is counted, a quantity corresponding to this residual reduction must be diminished from the gross value received. From the curve can be seen that the plasma vitamin C concentration of 0.80 mg% corresponds to about 4.0 mg% concentration in the urine, of which the share of the ascorbic acid is about 3.0 mg%. If the quantity of the urine excreted in twenty-four hours is assumed to be 1500 ml., then the twenty-four-hour excretion of the ascorbic acid is 45 mg. Respectively, the plasma vitamin C concentration of 1.0 mg% is corresponding to an excretion of about 68 mg. in twenty-four hours.

The renal threshold value of the ascorbic acid varied from 0.75 to 1.40 mg% in different individuals.

The specific gravity of the urine was on an average 1023 and did not depend on the ascorbic acid standard.

The pH of the urine was on an average 5.8 determined according to my own indicator-paper method (U u s p ä ä, formerly N y b e r g '26, '30).

CHAPTER VI

ON THE VITAMIN A AND C SERVICE OF THE SOLDIERS DURING THE WAR

It is obvious that in the wooded soil of Finland and in the latitudes of Finland where in the winter the twilight never fully disappears even at noon, the soldier may not be blind in dim light. Already slight disturbances in the dark adaptation of which those concerned need not be conscious themselves, may bring the soldier f.ex. on a nightly raider in a worse position compared to the in the twilight more sharp-eyed enemy. Likewise a sentinel must be keen-eyed even in dim light in order to be able to do his important duty. In a war of to-day a soldier must possess all the human abilities undiminished.

The mobilisation of a small nation is rather total. The army includes a considerable part of old age classes. The ability of dark adaptation decreases somewhat with the age (Lindqvist, l.c.). Vitamin A appears to have, even according to my own tests, an improving effect also on normal but somewhat high threshold values (see Chapter II:3). Bathelder's tests ('34) on rats indicate that a rich vitamin A intake increases the vitality of the tissues and decreases the effects of senility. The effect of a good vitamin A standard is not limited thereto that the regeneration of the visual purple is good, but the controlling effect of vitamin A covers all the area of the epithelial tissue (Wolbach and Howe, Pillat, l.c.). Vitamin A increases the local resistance of the epithelia, among others that of the mucous membranes, against infection (Green and Mellanby '28, Green *et al.* '31, Simola and Brunius '33). And the remarkable part of the infections as the cause of losses is known from all wars, also from those carried on in Finland (Soininen '42, Kokko '45).

As hemeralopia can develop very quickly, even in the course of a few days, and as the soldiers thus, most often without knowing of it themselves, in a short time might become more limited for military tasks, special attention should be paid during the period of war

to the vitamin service of the soldiers. In winter time the vitamin A standard of the soldiers ought to be raised by distributing either liver preparations or vitaminized butter at opportune intervals. A high vitamin A standard is a good protection, not only against hemeralopia, but also against local infections.

Although there does not seem to be any corresponding physiological ability as susceptible to decrease of the vitamin C standard as dark adaptation is to decrease of the vitamin A standard, yet the importance of the normal vitamin C reserves is very great for the soldiers.

Bartlett, Jones and Ryan ('40) have stated that in surgical patients the vitamin C requirements have increased after operations which should be due to the consumption of same in the tissue repair processes. Bartlett *et al.* ('42, '43) showed also that the resistance of the scar tissue against mechanical elongation had decreased in low vitamin C standard. Also Lund and Crandon ('41) are of the opinion that the vitamin C reserves of the surgical patients schould not decrease too much. The healing of fractures also appeared to be accelerated by the vitamin C intake (Glangrasso '38). Skin-transplantations healed quicker in patients whose vitamin C standards were good (Evans '43). In traumatic and hemorrhagic shock intravenous ascorbic acid injections had a favourable effect (Stewart, Learmont and Pollock '41, Ungar '43). In fatigue condition the vitamin C content of the adrenals and the liver of the test animals decreased considerably (van Eekelen and Kooy '34). It seems as if a good vitamin C standard would have a favourable effect on healing in such cases of illnesses which are common in the circumstances of war.

Indeed, Dahlberg, Engel and Rydin ('44) could not observe any adverse effects as a result of even a considerable saturation deficit of vitamin C. The vitamin C intake on the other hand did not affect, it seemed, the occurrence frequency of the infectious diseases. The test time in the investigation was, however, comparatively short, and the vitamin C standard of the test persons before the test was apparently good. In three months there is truly time enough that a considerable saturation deficit may be brought about, and the vitamin C might even totally disappear from blood plasma (Crandon *et al.*, l.c., Rietschel and Mensching, l.c., etc.). But the reactions of the normal mesenchyma to vitamin C deficiency are slower. The "incubation time" of the clinical manifestations is more than 7 months (Tobler, Fox *et al.*). The latent changes, it is true, might develop quicker even in a human being, and the decrease of the vitamin C standard need not be as great as in manifest scurvy. Similarly, in vitamin A deficiency hemeralopia develops quicker, and due to a slighter decrease of vitamin A standard, than xerophthalmia. On the other hand the experience shows that even a normal vitamin C standard does not hamper the invasion of bacteria.

Neither is the ascorbic acid any bactericide substance. Yet, the dependence of the local sulphanilamid effect on an certain vitamin C standard could be established (J o n e s *et al.* '43).

The circumstances of the war are much more severe than those in peace time tests, and the war diseases and injuries often are of most serious kind. To get over them, with the least possible consequences, implies an optimal recovering ability of the organism. In severe traumata and illnesses even the side-diagnoses, among others hypovitaminoses, must be ascertained and attended in order to attain on optimal tissue activity. Numerous observations from previous wars indicate that there are included in the war circumstances more factors which dispose for the scurvy. Therefore, especial attention should be paid to the development of the soldiers' vitamin C standard during the war. An endeavour should be made to maintain a moderate vitamin C standard, principally through food but, should it not prove to be satisfactory, synthetic accorbic acid should be given.

SUMMARY

The aim of the present research was to study the vitamin A and C status in different groups of the Finnish population, especially in prisoners and soldiers. The material is divided into two parts, of which the first contains the results of measurements of dark adaptation in different groups of population, a total of 750 persons. The second part consists of results of determinations of ascorbic acid in the same groups, a total of 498 persons.

The *first part* of the study begins with a description of symptomatology of the vitamin A deficiency, dealing in particular with the occurrence of hemeralopia as an early symptom. The conclusion is reached that dark adaptation is essentially dependent upon the vitamin A standard and that it may, with certain reservations, be used in measuring the vitamin A standard.

In the measurements of dark adaptation the biophotometer of Jeans, Blanchard and Zentmire was used. In part of the cases the tests were made according to the original method of **Jeans** and others and the results were estimated according to the rules formulated by Saksela. The majority of the cases were tested by the original method modified by the author and the results were estimated both according to the rules of Saksela and to those of the author. These rules are based upon tests made on two control groups. The results of the tests on the one group, consisting of 100 primary school pupils, were treated statistically. The other group consisted of 50 healthy persons whose vitamin A standard was found to be good.

Of the 118 male prisoners examined in the summer of 1939 at the Karvia Reserve Prison, the dark adaptation was normal in 20.3 % and subnormal in 74.6 % of the cases, 5.1 % must be considered as border cases.

Examinations made in early spring 1941 gave a slightly more

favourable picture of the vitamin A condition of the prisoners. At that time 50 % had normal dark adaptation, 32.8 % subnormal adaptation and 17.2 % were border cases. The vitamin A condition was somewhat poorer in April—June 1945 in female prisoners, 38.7 % of whom had normal and 58.1 % subnormal dark adaptation and 3.2 % were border cases. Of the 11 prisoners examined in the Central Prison of Helsinki, 3 had normal and 6 subnormal dark adaptation. Night blindness caused by vitamin A deficiency appeared in the years 1939, 1941 and 1945 to a considerably greater extent in the prisoners than in the free population, being on a diet of their own choice. Especially the long working hours in the summer appeared to have increased the prisoners' need of vitamin A. In addition to hemeralopia, other eye symptoms were also manifested in the prisoners, such as lachrymation, stinging sensation in the eyes, fatigue from reading, especially in failing light, as well as being dazzled in bright light.

That the weakening of the dark adaptation and other eye symptoms of the prisoners were caused by hypovitaminosis-A is shown by the results of the vitamin A treatment. 10,000 I.U. of vitamin A daily within a period of 1—7 weeks very greatly reduced the threshold values in eight subjects and eliminated the eye troubles appearing when reading. A rich intake of vitamin A seemed to improve the threshold values of normal subjects as well, even of those aged over 35 years and increased the ability of the eye to see in dim light.

The results confirm the observation that in deficiency hemeralopia the sight in twilight may be disordered already in the first stage of dark adaptation and that also the function of the cones seems to depend on the effect of vitamin A. Light adaptation also seems to be disturbed through a deficiency in vitamin A, a subjective symptom of this being a dazzling of the eyes by bright light.

The quantities of vitaminized margarine and other food-stuffs included in the prison dietary and containing about 1,500 I.U. of vitamin A in the summer and about 1,200 I.U. in the winter and about 1,000 I.U. of carotene do not appear to have been sufficient to meet the vitamin A requirements of the prisoners. The extension of the rights of prisoners, stipulated in 1945, to receive additional

and more varying food, e.g. fats, in food parcels sent from outside the prison, seems therefore to have been dictated by real necessity.

The military material chiefly comprises cases examined in the years 1942—43 during the last war in the town of Käkisalmi. The vitamin A status of the soldiers of the coast-defence regiments of Ladoga, who during the war had perhaps a closer contact with the civilian population than other troops at the front was on the basis of adaptation tests, in the first half of the year 1942 clearly poorer than that of the civilian population at the rear, and also worse than that of the frontline-troops during the previous war, according to the investigations of Simola and Saksela. Of the whole material comprising 245 cases, 68.6 % were normal, 22.4 % subnormal, and the dark adaptation of 9.0 % had to be considered a borderline value. In the latter half of 1942 a distinct improvement of the adaptation results appeared, evidently due in part to the increased content of vitamin A in milk and butter, and in part to the food supplies given to the soldiers by the civilian population returning to their homes.

The adaptation tests performed among the population of Karvia parish (153 cases) showed that the vitamin A standard was remarkably good, especially among the primary school pupils in the church village of Karvia even as late as early spring 1941. 89 % of the school children had normal and only 7 % clearly subnormal dark adaptation. In the group of adults the dark adaptation was normal in 75.5 % and subnormal in 24.5 %.

The vitamin A standard of the displaced persons from Karelia returning to their homes seemed to be somewhat lower: normal in 63 %, subnormal in 29.6 % and border cases 7.4 %.

In the *second part* of this study the characteristics of a normal vitamin C standard in man were first dealt with. Attempts were made to find the norms of estimation of the vitamin C standard on the basis of plasma ascorbic acid values. After some deliberation 0.20 mg% was accepted as a limit, and values below that line were considered as indicating a subnormal vitamin C standard. 0.60 mg% or, with certain reservations, 0.40 mg% was taken as the limit below which the plasma ascorbic acid values do not show a physiologically fully sufficient vitamin C standard. On the basis of comparison between the concentration of ascorbic acid in blood

plasma and urine, 0.75 mg% was stipulated as the lower limit of the optimal vitamin C standard.

For the purpose of determining the ascorbic acid in the blood plasma, Tillmans' dichlorphenol-indophenol method modified by Farmer and Abt was used. Attempts were made to define the vitamin C condition of the different groups of the population on a basis of average values calculated from the figures obtained by this method.

50 % of the prisoners examined in the Karvia Reserve Prison in early spring of 1941 (77 cases) had a subnormal (inadequate) vitamin C standard and 41.3 % had ascorbic acid concentration in the plasma indicating an inadequate rather than adequate vitamin C standard. Only 8.7 % had a concentration above 0.40 mg%.

According to the examinations made in 1940—43 of soldiers (283 cases), 65.7 % had a subnormal ascorbic acid standard and 11 % at the most had a satisfactory level. 23.3 % had a plasma ascorbic acid concentration which was subnormal rather than satisfactory. The ascorbic acid content remained subnormal during the whole first half of the year, being 0.08 mg% in 1942 and 0.17 mg% in 1943. The monthly minimum (0.04 mg%) of 1942 was recorded in March, the maximum (0.49 mg%) in October. Zero values were found in 32 cases (16.8 %) in 1942, in 1943 only once. The poor vitamin C standard in soldiers in the first half of 1942 was due to the fact that the potatoes had frozen and thawed again and different kinds of food prepared of them had a very low ascorbic acid content.

42.3 % of civilians living on a diet altogether of their own choice (123 cases) had a subnormal ascorbic acid content in the blood plasma and in further 26 % it was rather insufficient. 11.4 % had an uncertain adequate vitamin C standard. In only 20.3 % the ascorbic acid concentration of the plasma was above 0.60 mg%. In the first half of the year none of the subjects had a normal ascorbic acid standard. There were 7.1 % uncertain normal cases. 66.1 % were subnormal and uncertain subnormal 26.8 %. Also in the latter half of the year almost one half (47.8 %) of the subjects had not received sufficient vitamin C. The different vitamin C standard in people in the latter half of the year evidently depends on their dif-

ferent alimentary habits and partly on their different requirements of vitamin C.

A low ascorbic acid content in the blood plasma in the first half of the year was characteristic of all groups of the population examined. The ascorbic acid values of the civilian population made a sharp improvement in August, those of soldiers in 1942 did not improve until October. In prisoners, no changes in the ascorbic acid values of the plasma were seen in August. This difference was due to lack of new potatoes and other fresh vegetables, rich in vitamin C, in the standard diet. In mass-provisioning general use of the new potato crop is not made until the end of September or the beginning of October. Therefore, in such provisioning, an adequate supply of vitamin C was of two months' shorter duration and its effect on the vitamin C standard remains considerably weaker than in the civilian population. This variation in the vitamin C standard in the Finnish population during different seasons cannot be considered a normal and positive phenomenon from the point of view of public health.

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CORRIGENDA

Page 7;	line 32:	hypovitaminosis-B_1) ..	read	hypovitaminosis B_1
„ 17,	„ 29:	grils	„	girls
„ 22,	„ 26:	adaption	„	adaptation
„ 22,	„ 31:	test	„	tests
„ 37,	„ 30:	border-line	„	borderline
„ 40,	„ 2:	Dark Adaptation Tests	„	Results from Dark Adaptation Tests
„ 57,	„ 21:	oxidized	„	acidulated
„ 64,	„ 10:	0.20 mg%	„	0.22 mg%
„ 69,	table 14,	column 1940, VIII 0.19	„	VII 6 0.19
		VII 6 0.07	„	VIII 3 0.07
		IX 3	„	IX
„ 83,	line 16:	accorbic	„	ascorbic
„ 90,	„ 35:	Proc.Soc.Exp.Biol.Med.	„	Proc.Soc.Exp.Biol. & Med.